The Role of Functional-traits in the Response of Tropical Forests to Global Change

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I dedicate this thesis to my father Dr. Patrick F. O'Brien, the man who taught me about integrity.

Contents

General Introduction

Tropical forests are important as biodiversity hotspots and for providing global ecosystem services (Myers et al 2000; Malhi et al. 2009; Saatchi et al. 2011). However, they are threatened by logging, land-use conversion and climate change (Walsh & Newberry 1999; Chazdon 2003; Reynolds et al. 2011). In this thesis, I examine the importance of functional-trait diversity of rain-forest trees as insurance against climate change and in the recovery of primary forest species after logging.

Tropical forest value

Tropical forests cover 5% of the Earth's land area, representing 44% of the Earth's forest cover (FAO 2011). Historically, tropical forests have been heavily used as a source of timber products. However, their realized importance is in more sustainable services. They are a large global carbon sink which sequesters an estimated 1.19 Pg C $year^{-1}$ (Pan et al. 2011). They are an important part of the global water cycle, and they can provide evaporative cooling to mitigate warming (Huntington 2006; Bonan 2008). Furthermore, they represent some of the most diverse systems in the world (Myers et al. 2000). For example, the Sundaland island region in Southeast-Asia is estimated to contain 25,000 plant species of which 15,000 species are endemic (Myers et al. 2000). However, logging continues throughout much of the tropics in conjunction with a rapidly changing climate which leaves the future conservation of these forest systems in question.

Maintenance of diversity

The mechanisms underlying the coexistence of such high numbers of plant species in a limited geographic area, such as that found in tropical forests, remains

one of the most prominent unanswered questions in ecology. Numerous theories have been proposed to explain this high diversity but a generally recent unifying framework has been proposed which distinguishes between equalizing and stabilizing forces (Chesson 2000). This framework allows for systems to be viewed in terms of the magnitude of the differences in equalizing forces and the strength of the stabilizing forces. Therefore, systems can distribute along a continuum of these two axes (Adler et al. 2007; HilleRisLambers et al. 2012).

Neutral theory

At one end of this continuum, neutral theory proposes species are equal and only random drift affects coexistence (Hubbell 1986; Hubbell 2001). This theory is highly unlikely as measurable differences in species traits are commonly observed. An alternative formalization of this theory asserts that species differ in their traits, but trade-offs in traits produce equality in fitness. Again, this scenario is implausible as deviations in traits would cause the balance in neutral fitness to fail (Turnbull et al. 2008; Purves & Turnbull 2010). However, within the framework of equalizing and stabilizing forces small trait differences would require weak stabilizing forces to prevent competitive exclusion and maintain diversity, which would closely follow neutral theory (Adler et al. 2007). Tropical forests are on such system in which proposed fitness differences are small, requiring small niche differences.

Niche differences

Stabilizing forces are recognized as niche differences which alter the competitive advantage of species (Levine & HilleRisLambers 2009). Niche partitioning prompts frequency-dependent interactions where by population growth rates increase when rare (Chesson 2000; Levine & HilleRisLambers 2009;

HilleRisLambers et al. 2012). However, in long-lived organisms population growth rates are difficult to quantify, and niche differences are therefore often assumed from functional traits which produce life-history trade-offs (Kraft et al. 2008; Baraloto et al. 2012). In tropical forests, a great deal of work has identified some of the important niche axes promoting species coexistence including small-scale species partitioning along light and microclimatic gradients (Brown & Whitemore 1992; Brown 1993; Davies et al. 1998; Philipson et al. 2012), landscape-level partitioning across soil fertility gradients (Webb & Peart 2000; Paoli et al. 2006; Katabuchi et al. 2012) and numerous biotic interactions (Janzen 1970; Connell & Slatyer 1977; Peay et al. 2009; Bagchi et al. 2010; Eichhorn et al. 2010). However, disturbances such as logging and climate change affect the processes that promote species coexistence both directly, by physically disrupting interactions and causing extinctions, and indirectly, by altering species distributions and interactions. The direction and magnitude of plant response to human and climatic disturbance is of import for the conservation and restoration of tropical forests (Chazdon 2003).

Climate change and rainfall variability

Climate change induced alterations to global precipitation patterns will have substantial effects on tropical forests (Huntington 2006; Williams et al. 2007). These shifts in precipitation patterns are characterized by an intensification of extreme rainfall events, increased variability in rainfall patterns and more frequent and severe droughts driven by an intensification of El Nino Southern Oscillation (ENSO) events (Timmermann et al. 1999; Walsh & Newberry 1999; Huntington 2006). ENSO events are defined by a slackening of the trade winds causing a warming in the eastern Pacific Ocean and high air surface pressure in the western Pacific Ocean. These events cause below average rainfall in the tropical forests of the Amazon, Central

America and South-east Asia (Curran et al. 1999; Walsh & Newbery 1999; Engelbrecht et al. 2007; Lewis et al. 2011). This intensification in the severity and frequency of ENSO related droughts may impact distributions of species and cause extinctions by introducing novel climate zones (Williams et al. 2007).

Drought as a stabilizing force

Drought influences species distributions in seasonally dry and everwet tropical forests at both landscape- and local-scales (Gibbons & Newbery 2003; Slik 2004; Engelbrecht et al. 2007; Comita & Engelbrecht 2009). However, in order to address drought in terms of species coexistence, we need to be define it in terms of its simplest characteristic, a reduction in water as a resource. Within this definition, a trade-off can be formulated in which fast growth rates under high water availability (generating fitness differences) comes at the cost of poor competitive ability for water when water availability is low (stabilizing niche differences). These faster growing species would be competitively dominant under wet conditions when water is not a limiting resource but would decrease in fitness under drought conditions. This scenario could occur spatially in which drought tolerant species would fill empty niche space not used by fast growing species or temporally in which seasonal and supra-annual droughts allow persistence of slow growing species but better resource competitors (i.e. storage effect; Chesson & Warner 1981; Adler et al. 2006; Angert et al. 2009).

The differential response of species to drought is dependent on differences in functional traits. The response of plants to drought is often summarized into two strategies for enduring drought stress: avoidance and tolerance (McDowell et al. 2008; Markesteijn & Poorter 2009). Drought avoiders reduce transpiration through

stomatal closure (Brodribb & Holbrook 2003), use deep roots to access water reserves (Chaves et al. 2002) and shed leaves to reduce respiring biomass (Brodribb et al. 2003; McDowell et al. 2008; Bartlett et al. 2012). Drought tolerators maintain stomatal conductance for continued growth under dry conditions using stored carbon to maintain cell turgor pressure and vascular integrity (Hsiao 1973; McDowell et al. 2008; Zwieniecki & Holbrook 2009; Bartlett et al. 2012). The lack of adaptation to drought may result in species extinction under future climate change induced drought.

Experimental setting - Borneo

The everwet tropical forests of Malaysia Borneo provide an ideal location to examine the effects of both direct human disturbance and climate change induced drought for multiple reasons. First, it is a biodiversity hotspot in the tropics (Myers et al. 2000) with a primary forest canopy dominated by trees from the family Dipterocarpaceae (dipterocarps), many species of which are endemic to Borneo (Wood & Meijer 1964; Whitemore 1984; Ashton 2004). Second, dipterocarps in Borneo exhibit a unique reproductive strategy, which could potentially inhibit seedling recruitment after logging. Dipterocarps rely on synchronized mast fruiting events for the majority of their regeneration (Sakai et al. 2006). These events occur unpredictably on supra-annual cycles but commonly coincide with ENSO events (Ashton et al. 1988; Curran et al. 1999; Sakai et al. 2006). Furthermore, many dipterocarp species produce recalcitrant seeds (i.e. desiccation sensitive), which may make seeds sensitive to intensified droughts. Third, extensive areas of primary forest in Borneo was degraded by logging and/or converted to oil palm plantation (Reynolds et al. 2011). The rapid conversion to oil palm has led to an increased focus on conservation and restoration of logged forests (Berry et al. 2011; Edwards et al. 2011). Therefore, understanding the processes operating to promote species diversity

in logged forests and the effect of climate change induced drought on tree survival is necessary for conservation and restoration.

Conclusion

In this thesis, I examine the effects of disturbance on species coexistence using two approaches. First, I perform an observational study to assess the mechanisms that promote diversity in logged forests. Second, I use experimental manipulations to examine drought as a potential niche axis in the tropical forests of Borneo. For both methods, I examine a range of species in order to identify variation in key traits, which provide tolerance to and recovery from disturbance. Plant response to human disturbance and climate change will depend on the rate and magnitude of the change, trait variability and interactions with other abiotic and biotic factors (Clark et al. 2012; Buckley & Kingsolver 2012), and if species vary in their traits, then we assume their response to disturbance will also vary.

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Summary

In **Chapter 1**, I use a landscape-scale mast fruiting event to examine the state of regeneration in a logged forest and the processes that promote diversity. My findings conclude that although dispersal is the major limiting factor to forest recovery in a logged forest, the processes that function to maintain diversity in primary forests also function in logged forests to promote diversity. Rare seeds are predated less than abundant seeds, and con-specific seedlings of mature trees have greater mortality than hetero-specific seedlings. These processes had yet to show a negative density-dependent effect on recruitment because the small seedling size meant competition for resources had not begun. My results indicate that management to promote seedling diversity is likely beneficial in restoring logged forests.

In **Chapter 2**, I test whether rainfall variability has an impact on seed germination and early seedling growth across a wide range of seed masses. As climate change is altering precipitation cycles, the rainfall frequency and intensity will play a greater role in shaping species distributions. I show that germination of large seeded species was less susceptible to fluctuations in rainfall, but larger seedlings had reduced growth without frequent rainfall. This result indicates that rainfall variability has the potential as a niche axis at the earliest stages of seedling establishment, and if climate change projections are correct, then this niche will play a greater role in shaping communities in the future.

In **Chapter 3**, I examine seedling response to experimental manipulations of two aspects of drought: water deficit and fluctuations in water availability. The results show that a variety of traits were important for drought resistance including below-ground biomass allocation under mild drought and physiological control under severe

drought. Species partitioned across a gradient of resistance to drought which suggests that species may distribute across topographic gradients and landscapes as a response to water availability. Again, these results have increased importance for understanding the effects of climate change scenarios on species distribution and community assembly.

In **Chapter 4**, I test the hypothesis that greater non-structural carbohydrate concentrations in seedlings will improve resistance to drought. I use a novel experiment to manipulate within species non-structural carbohydrate concentrations without causing differences to within species size. In other words, before the drought, seedlings of the same species were approximately the same size but were either enriched or depleted in non-structural carbohydrates. The results show that within a given species individuals with greater non-structural carbohydrate concentrations survived longer than those with lower non-structural carbohydrate concentrations. Among species comparisons also showed a strong correlation between the amount of non-structural carbohydrate concentrations and days to death. Although the functional role of non-structural carbohydrates in drought resistance was not elucidated, the results directly show more non-structural carbohydrates provide a benefit to seedlings under drought stress through prolonged survival.

Zusammenfassung

In **Kapitel 1** verwende ich ein Mastfrucht-Ereignis auf regionaler Ebene, um den Regenerierungszustand eines durchforsteten Waldes und die Prozesse, welche die Artenvielfalt erhalten, zu untersuchen. Meine Ergebnisse kommen zum Schluss, dass, obwohl die Samenverbreitung der hauptsächliche limitierende Faktor für die Regeneration eines durchforsteten Waldes ist, die Prozesse, welche die Artenvielfalt in durchforsteten Wäldern erhalten, dieselben sind wie in Primärwäldern. Seltene Samen werden seltener gefressen als häufige Samen, und Keimlinge haben in der Nähe gleichartiger Bäume eine höhere Mortalität als in der Nähe andersartiger Bäume. Es muss allerdings erst noch gezeigt werden, dass diese Prozesse einen dichte-abhängigen Effekt auf die Regeneration haben, denn die Konkurrenz zwischen Keimlingen ist gering, bis diese eine gewisse Grösse erreicht haben. Meine Resultate deuten darauf hin, dass ein gezieltes Management der Artenvielfalt von Keimlingen für die Wiederaufforstung durchforsteter Wälder vorteilhaft sein kann.

In **Kapitel 2** teste ich über einen grossen Bereich an Samengrössen, ob Niederschlagsschwankungen einen Einfluss auf die Keimung und das frühe Keimlings-Wachstum haben. Da der Kimawandel die Niederschlagszyklen verändert, werden Niederschlagshäufigkeit und -intensität bei der Artverbreitung eine grössere Rolle spielen. Ich zeige, dass Arten mit grossen Samen zwar weniger anfällig sind auf Niederschlagsschwankungen, welche sich vor der Keimung ereignen, aber dass grössere Keimlinge ohne regelmässigen Niederschlag ein reduziertes Wachstum haben. Dieses Ergebnis deutet darauf hin, dass Niederschlagsschwankungen als Nischenachse während der Etablierungsphase von Keimlingen funktionieren könnten

und dass diese Nische entsprechend des vorhergesagten Klimawandels eine grössere Rolle bei der Zusammensetzung zukünftiger Artgemeinschaften spielen wird.

In **Kapitel 3** untersuche ich die Reaktion von Keimlingen auf die experimentelle Manipulation zweier Aspekte von Trockenheit: Wassermangel und Fluktuation in der Wasserverfügbarkeit. Meine Resultate zeigen, dass für die Trockenheitsresistenz eine Vielzahl von Merkmalen wichtig ist, einschliesslich der unterirdischen Biomasse-Allokation unter leichter Trockenheit und der physiologischen Kontrolle unter schwerer Trockenheit. Pflanzenarten sind über einen Trockenheitsresistenz-Gradienten verteilt, was vermuten lässt, dass sich die Arten auf lokaler und regionaler Skala entsprechend der Wasserverfügbarkeit verteilen. Auch diese Resultate sind wichtig, um die möglichen Folgen verschiedener Klimawandel-Szenarien für die Artverteilung und die Zusammensetzung von Pflanzengemeinschaften zu verstehen.

In **Kapitel 4** teste ich die Hypothese, dass eine erhöhte Konzentration an nicht-strukturellen Kohlenhydraten die Trockenheitsresistenz von Keimlingen verbessert. Ich verwende ein neuartiges Experiment, um die Konzentration an nicht-strukturellen Kohlenhydraten von Keimlingen innerhalb einer Art zu manipulieren, ohne deren Grösse signifikant zu beeinflussen. Das heisst, vor der Trockenheit waren Keimlinge der selben Art ungefähr gleich gross, hatten aber einen erhöhten oder verringerten Anteil an nicht-strukturellen Kohlenhydraten. Die Resultate zeigen, dass innerhalb einer bestimmten Art Individuen mit einer höheren Konzentration an nicht-strukturellen Kohlenhydraten länger überleben als Individuen mit einer geringeren Konzentration. Vergleiche zwischen den Arten zeigen ebenfalls einen starken Zusammenhang zwischen der Menge an nicht-strukturellen Kohlenhydraten und der Lebensdauer eines Keimlings. Obwohl die funktionale Rolle nicht-struktureller

Kohlenhydrate bei der Trockenheitsresistenz nicht direkt erläutert wurde, zeigen diese Resultate, dass ein erhöhter Anteil an nicht-strukturellen Kohlenhydraten Keimlingen unter Trockenheits-Stress einen Vorteil bietet.

Chapter 1

Regeneration dynamics following a mast fruiting in a logged forest: evidence for the density-dependent mechanisms that could restore high diversity?

Michael J. O'Brien, Roman T. Kellenberger, Christopher D. Philipson, John Tay & Andy Hector

Photo courtesy of Roman Kellenberger

Abstract

Density dependent processes that maintain diversity in tropical primary forests are commonly altered after logging. We posed the question: Are the same processes that maintain species in primary forests operating to restore tree diversity in logged forest? We used a large regional-scale mast fruiting in Sabah, Borneo which had a high percentage of flowering trees to examine early regeneration dynamics in logged tropical forests. We analyzed seed and seedling mortality surrounding mature flowering trees in logged forest in order to assess mechanisms that promote diversity at dispersal, establishment and recruitment stages. Rare seeds had a lower probability of mortality than common seeds, due to increased death of con-specific seeds from fungal pathogens. Density increased seedling mortality but the effect was not significantly different between con- and hetero-specifics, although, around two of the mature tree species, a trend of lower mortality of hetero-specific seedlings was observed. After nearly two years, survivorship was quite high relative to other studies, and negative density dependent mechanisms were not evidently affecting overall seedling recruitment. However, processes that promote diversity were functioning at the seed and seedling stages within logged forests, and as the seedling cohort develops competitive processes which favor intra- over inter-specific mortality will likely begin affecting recruitment.

Keywords

Dipterocarps; Borneo; Mast fruiting; Species coexistence; Disturbance; Sabah Biodiversity Experiment; Dispersal limitation

Introduction

Primary tropical forests are highly diverse ecosystems. The Sunda region alone, which includes Borneo, is estimated to contain as much as 5.0% of the world's plant diversity (Myers et al. 2000). Density dependent mechanisms that suppress extinction of rare species, inhibit dominance of abundant species and mediate intra- over inter-specific competition (Chesson 2000) maintain high species diversity in primary tropical forests (Webb & Peart 1999; Harms et al. 2000; Webb et al. 2006; Comita et al. 2010; Bagchi et al. 2011). However, logging may disrupt these processes, which raises the question of whether the mechanisms that maintain species coexistence in primary forests still operate in secondary forests to help restore the primary forest diversity removed by logging (Wright 2002).

Regeneration processes in the aseasonal moist forests of Borneo may be particularly vulnerable to logging. This ecosystem has been heavily logged since the 1960's but recovery of the dominant family Dipterocarpaceae (dipterocarps) is slow because of the family's episodic mast seed production (masting events) and the intensity of logging in the area (Ashton et al. 1988; Appanah 1993; Curran et al. 1999; Berry et al. 2008; Reynolds et al. 2011). For example, an area of fire disturbed forest had few late successional species seven years after the fire due to the lack of a post-fire masting event (Slik et al. 2011). Masting events occur at irregular intervals making them difficult to predict and research (Ashton et al. 1988). Therefore, a limited body of work exists on the early establishment and recruitment phase of dipterocarp species (Webb & Peart 1999; Curran & Webb 2000; Maycock et al. 2005; Bagchi et al. 2010; Bagchi et al. 2011). A few studies have examined regeneration of primary forest trees in logged dipterocarp forest, but results have been contradictory probably due to variability in the size of the masting event, the severity of logging, the

length of the recovery period and differences in the reproductive biology of the species examined.

The forest recovery process is dependent on the size and severity of the disturbance (Chazdon 2003). Recovery time is inversely correlated with total timber extracted and directly correlated with distance from seed-producing trees (Chazdon 2003). Low diversity and abundance of remnant mature dipterocarps in a logged forest could lead to lower species and genetic diversity of subsequent seedling cohorts and the formation of patches of isolated single species patches centered about the mature remnant trees. Beyond the dispersal stage, the processes driving regeneration and recruitment patterns in a primary forest are often disturbed following logging (Curran & Webb 2000; Bagchi et al. 2011). As an example, density dependent mortality is a well-studied mechanism maintaining species coexistence in primary tropical forests (Webb & Peart 1999; Harms et al. 2000; Webb et al. 2006; Bagchi et al. 2011), but logging disturbance could either weaken or strengthen this process by altering invertebrate and vertebrate communities which influence seed predation and seedling herbivory (Curran & Webb 2000; Hautier et al. 2010; Bagchi et al. 2011).

A large-scale masting event in Borneo in 2010 (with >75% of mature trees estimated to have flowered; Kettle et al. 2011) provided an opportunity to investigate seed dispersal, establishment and recruitment near three mature dipterocarps in a logged forest. We used isolated mature dipterocarp trees to assess whether locally derived con-specific seeds and seedlings (individuals of the local mature tree) survived worse than hetero-specific seeds and seedlings from neighbours. We tested the effects of mature tree species identity, density of regenerating seeds and seedlings, distance to the mature tree and local abundance (proportion of local seedling community) on survival of con- and hetero-specific seeds and seedlings. We then

examined if recruitment between the seed and seedling stage was affected by density dependent mortality. We posed the following questions regarding regeneration and recruitment dynamics: (1) Do hetero-specific seeds invade isolated seed populations? (2) Do locally abundant seeds and seedlings near mature dipterocarp trees have greater mortality than locally rare individuals? (3) Do con-specific individuals of mature dipterocarps have greater mortality than invading hetero-specific individuals? (4) Does the relationship between total seed fall and seedling recruitment differ between con- and hetero-specific individuals?

Methods

Study site

The experiment took place in the Malua Forest Reserve (Malua), an area of logged forest which includes the Sabah Biodiversity Experiment (N05º05'20'' E117º38'32''; 102 MASL). This site is located ≈22 km north of Danum Valley Research Center in Sabah, Malaysia (Hector *et al*. 2011). Mean annual rainfall (SE) recorded in Danum Valley from 1986-2010 was 2848.5 (94.0) mm. The primary forest in this area is dominated by dipterocarps, but these species have been the main target of the logging industry. Malua was intensively logged more than two decades ago and, with the exception of the Sabah Biodiversity Experiment, re-logged between 2005-2010. The remaining forest is predominately secondary with dense patches of climbing bamboo but occasional mature dipterocarps can still be found. The vegetation is heterogeneous due to variation in the historical logging intensity and topography.

Experimental design

We chose three dipterocarp species (*Dryobalanops lanceolata*, *Parashorea malaanonan* and *Shorea parvifolia*) for the study (Table 1). We selected these species because they were available in Malua, are common species in the neighbouring primary forest at Danum Valley and vary in key traits (Table 1). We located three

Table 1 The germination and seedling establishment traits of the mature species used in this experiment. These values were taken from a shade-house experiment under approximately 5% direct sunlight transmission at the Sabah Biodiversity Experiment using seeds collected during the 2010 masting event (O'Brien, *in preparation*). All growth metrics were measured 60 days after germination.

	Dryobalanops lanceolata Burck	*Parashorea malaanonan* Merr.	*Shorea parvifolia* Dyer
Seed dry weight ± 95% CI (mg)	2638.0 ± 343.4	1935.9 ± 184.6	508.5 ± 21.1
Days to germination ± 95% CI	3.4 ± 0.3	11.0 ± 1.0	6.8 ± 1.1
Days to leaf out ± 95% CI	22.6 ± 1.4	53.7 ± 5.7	17.4 ± 1.0
Leaf area ± 95% CI (cm^2)	211.5 ± 13.8	73.2 ± 9.5	81.5 ± 4.7
Height ± 95% CI (mm)	306.1 ± 10.3	141.3 ± 9.7	176.2 ± 6.9
Root depth ± 95% CI (mm)	173.1 ± 7.8	144.4 ± 10.1	151.4 ± 8.5

mature trees (i.e. greater than 50 cm diameter at breast height and producing seed) of each species based on the following criteria: 1) free of stem and crown damage; 2) isolated by greater than 100 m from mature con-specific dipterocarps and at least 15 m from mature hetero-specific dipterocarps and 3) were at least 20 m from topographic disturbance (e.g. old logging roads and skid trails). In order to investigate the effect of distance from adult dipterocarps on seedling mortality, we placed plots of 2 x 2 m at 2, 6 and 18 m from the mature stem. Plots were laid out on three axes radiating from the mature tree at 0°, 120° and 240° azimuths in order to

prevent directional biases in seed fall from factors such as wind and slope. This gave nine plots at each of three mature trees from the three species for a total of 81 plots. We measured slope and aspect once for each plot. At each census, we measured canopy openness with a convex spherical densiometer (Forestry Suppliers Inc., Jackson, MS, USA).

Seed and seedling census

A landscape-level masting event began in Malua in late July 2010 with seed fall finishing in early September 2010. The initial seed and seedling census began on August 11th, 2010 during the peak of the event. A second census began September 13th, 2010 after the masting event had concluded in the area. Censuses continued approximately every three months thereafter with the last census occurring in April 2012, 20 months after the initial census.

At each census, we identified all individuals to species, tagged them (using numbered plastic chopsticks to mark seeds) and recorded whether they were alive or dead. We further classified each individual as either seed (prior to root formation and stem growth) or seedling. Where possible we recorded the cause of death (e.g. pathogen, vertebrates, invertebrates, etc.).

Analysis

In order to assess the mechanisms driving seed and seedling mortality, we analyzed mortality with generalized-linear mixed models with a binomial variance function and a complimentary log-log link function, which accounts for auto-correlation in the repeated samples and the unequal resample intervals. We performed this mortality analysis separately for seeds and seedlings to distinguish processes

acting at each ontogenic stage. The response variable was the proportion of dead con- and hetero-specific individuals in each plot at each census weighted by the total number of individuals still alive at the end of the previous census. A random intercept term for each plot and census was used to account for spatial and temporal variation inherent in the experimental design. We fit mature tree species identity, seedling density, distance from mature seed tree, canopy openness, local abundance (proportion of both con- and hetero-specific individuals within a plot) and relatedness to mature tree (whether a con- or a hetero-specific) as explanatory variables to identify the mechanisms influencing mortality. An offset was used to scale parameters to depict mortality on weekly (seeds) or yearly (seedlings) timescales. We used the Bayesian Information Criterion (BIC) for model selection.

To assess the causal mechanisms of mortality, we analyzed total mortality (counts) as a function of mature tree species, mechanism (e.g. pathogen, vertebrate, etc.) and relatedness to the mature tree (con- or hetero-specific) for seeds after week 18 (when all seeds had either become seedlings or died) and for seedlings after week 89, the final census. We used a Poisson distribution with a log link function and a random intercept for plot to account for geographic variation.

In order to assess the effect of seed fall density on recruitment, we modeled the relationship between the number of total seeds counted from census one and two and total seedling recruitment of con- and hetero-specific individuals for each mature tree species at the final census. Both variables were transformed with $\log_{10}$ plus one to make a linear relationship. We assumed that a slope of less than one implies that negative density dependence was affecting recruitment (Harms et al. 2000). In order to assess mortality patterns through time, we plotted the mean proportion of

individuals still alive near each mature tree species for con- and hetero-specific individuals at each census.

The glmer function in the lme4 library (Bates & Maechler 2011) of the R statistical software version 2.13.2 (R Development Core Team 2011) was used for mortality analysis and the arm package (Gelman & Hill 2007) was used in calculating 95% confidence intervals (CIs). The details of the CIs and the *p-value* estimation are located in the supplement.

Results

Hetero-specific invasion

For the majority of plots (90.1%) the seed community was dominated by con-specifics of the mature tree. Hetero-specific seeds were found in plots at 2, 6 and 18 m (Fig S1) and seeds of ten different species were identified. The mean number of species (SE) found near any mature tree was 2 (0.22). The mean (SE) number of hetero-specific individuals invading a plot was 19 (6) seeds, and there were 8 plots in which hetero-specific seeds outnumbered con-specific seeds (3 plots near a mature *D. lanceolata* and 5 plots near a mature *S. parvifolia*). Overall, hetero-specific individuals comprised from 0-83% of a plot.

Seed mortality

Distance from mature tree and seedling density explained little variability in mortality of seeds and were dropped from the model (determined by BIC). Seed mortality was influenced by the identity of mature tree species, relatedness to mature tree (whether a con- or a hetero-specific), their interaction and local abundance. Mortality was positively related to local abundance for all three mature tree species ($\beta_{abundance} = 0.65$,

95% CI: 0.27 – 1.04, $p < 0.0001$; Fig 1). *P. malaanonan* and *S. parvifolia* had significantly lower seed mortality than *D. lanceolata* ($\beta_{species}$ = -0.78, 95% CI: -1.1 – -0.44, $p < 0.0001$ and $\beta_{species}$ = -0.50, 95% CI: -0.84 – -0.16, $p < 0.004$; Fig 1). Con- and hetero-specific mortality was not significantly different around *D. lanceolata* and *P. malaanonan* ($\beta_{conhet*lanceolata}$ = -0.69, 95% CI: -1.4 – 0.05, $p < 0.08$ and $\beta_{conhet*malaanonan}$ = -0.13, 95% CI: -0.8 – 0.5, $p < 0.68$, respectively), but near *S. parvifolia* hetero-specific seed mortality was significantly greater than con-specific mortality ($\beta_{conhet*parvifolia}$ = 0.80, 95% CI: 0.6 – 1.1, $p < 0.0001$).

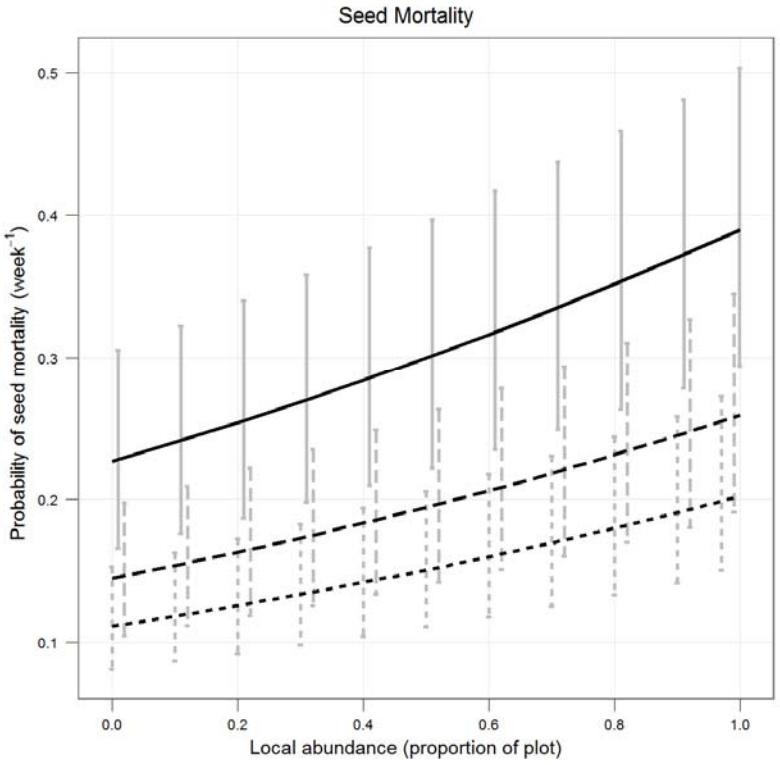

Fig 1 The probability of seed mortality per week (±95% CIs) as a function of local abundance in a plot. Local abundance was calculated as the proportion of con- and hetero-specific seedlings comprising the total plot density. Rare seeds in a plot have a lower probability of mortality. Lines represent model estimates (*D. lanceolata*: solid line; *P. malaanonan*: small-dashed line; *S. parvifolia*: long-dashed line).

Only model estimates for con-specific seedlings are presented for readability, as there was only a significant difference between on- and hetero-specific mortality near *S. parvifolia* trees.

Mortality of seeds after the first 18 weeks was driven by fungi, invertebrates and vertebrates, but the magnitude of the effect differed for con- and hetero-specifics (Fig 2). Con-specific mortality from fungal pathogens was significantly higher than hetero-specific mortality (β_{conhet} = 0.68, 95% CI: 0.17 – 1.13). All other mechanisms did not vary significantly between con- and hetero-specific mortality (Fig 2). Additionally, all other mechanisms of con-specific mortality were significantly lower than fungal pathogens ($\beta_{treefall}$ = -0.96, 95% CI: -0.2 – -1.6; $\beta_{invertebrate}$ = -0.81, 95% CI: -0.5 – -1.1 and $\beta_{vertebrate}$ = -0.39, 95% CI: -0.1 – -0.7; Fig 2). There were no differences in the mechanisms near different mature tree species.

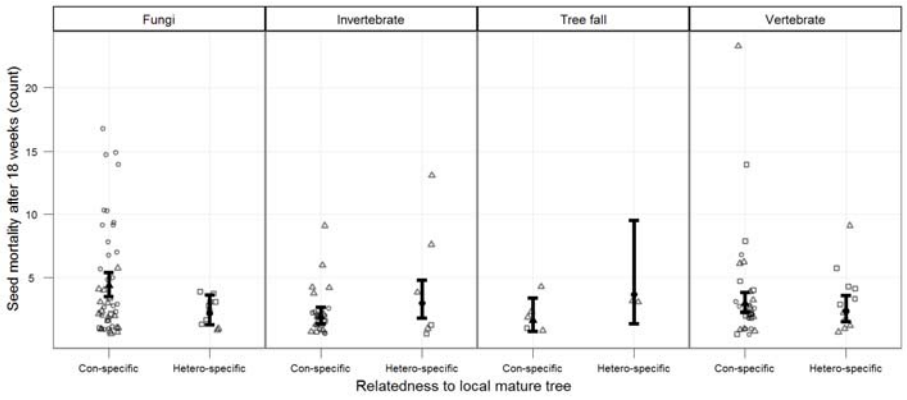

Fig 2 The total counts of seed mortality (±95% CIs) from the first 18 weeks as a function of observed mechanism, and whether the seed was a con- or hetero-specific in the plot. Con-specific seeds (closed triangles) had greater mortality from fungal pathogens than hetero-specific seeds (closed circles). The grey points in the background represent observed counts in each plot near each of the three mature species (*D. lanceolata*: open squares; *P. malaanonan*: open circles; *S. parvifolia*: open triangles) and are jittered for readability.

Seedling mortality

Both distance and local abundance explained little variability in seedling mortality and were dropped from the model (determined by BIC). The relationship between density and seedling mortality varied among mature tree species. Seedling mortality around *D. lanceolata* and *P. malaanonan* was positively related to density ($\beta_{density*lanceolata}$ = 0.04, 95% CI: 0.004 – 0.05 and $\beta_{density*malaanonan}$ = 0.08, 95% CI: 0.07 – 0.1, respectively) while around *S. parvifolia* seedling mortality showed a negative relationship with density ($\beta_{density*parvifolia}$ = -0.01, 95% CI: -0.02 – -0.002; Fig 3). Canopy openness was non-significant in explaining mortality (β_{canopy} = -0.02, 95% CI: -0.05 – 0.01), but density and canopy openness interact such that seedling mortality is greater with higher densities under more open canopies ($\beta_{density*canopy}$ = 0.002, 95% CI: 0.001 – 0.003; Fig 3). To test which species was driving this interaction, we systematically removed each mature tree species and compared the full model with the interaction to a model without the interaction using BIC. The model with the interaction always performed better when *S. parvifolia* was in the model, and if *S. parvifolia* was removed, then the model without the interaction performed better. Therefore, the density and canopy openness interaction was driven by the presence of *S. parvifolia* (Fig S2).

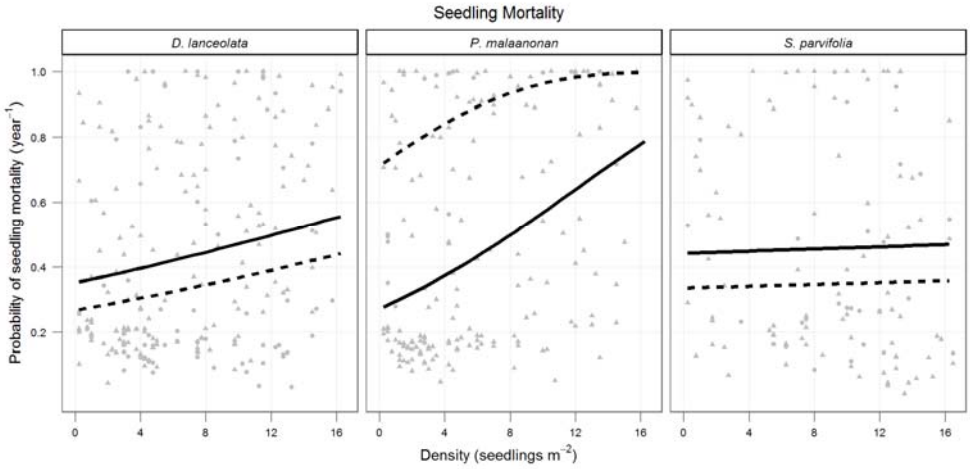

Fig 3 The probability of seedling mortality per year as a function of total seedling density for con-specific seedlings of the mature tree species (triangles and solid lines) and hetero-specific seedlings (circles and dashed lines) calculated at a mean canopy openness of 6%. Both *D. lanceolata* and *P. malaanonan* mortality significantly increased with density. Con-specific seedlings of mature *D. lanceolata* and *S. parvifolia* trees had higher mortality than hetero-specific seedlings while the opposite was true of conspecific individuals near mature *P. malaanonan* trees. The grey points represent the proportion at each plot in each census.

Mortality of con- and hetero-specific seedlings was dependent on the species of mature tree (Fig 3). Hetero-specific mortality was significantly lower around *D. lanceolata* and *S. parvifolia* ($\beta_{conhet*lanceolata}$ = -0.33, 95% CI: -0.57 – -0.1 and $\beta_{conhet*parvifolia}$ = -0.36, 95% CI: -0.46 – -0.25, respectively) and significantly higher around *P. malaanonan* ($\beta_{conhet*malaanonan}$ = 1.37, 95% CI: 0.9 – 1.9; Fig 3).

Seedling mortality was driven by tree fall and trampling from large vertebrates but was significantly higher for the latter ($\beta_{vertebrate}$ = 1.7, 95% CI: 1.4 – 2.0; Fig 4). *S. parvifolia* also had significantly higher mortality across both mechanisms ($\beta_{parvifolia}$ = 1.0, 95% CI: 0.4 – 1.6; Fig 4). These results only account for identifiable mechanisms, random individual morality of unknown causes occurred at each census. Con- and hetero-specifics did not differ in mechanisms driving mortality.

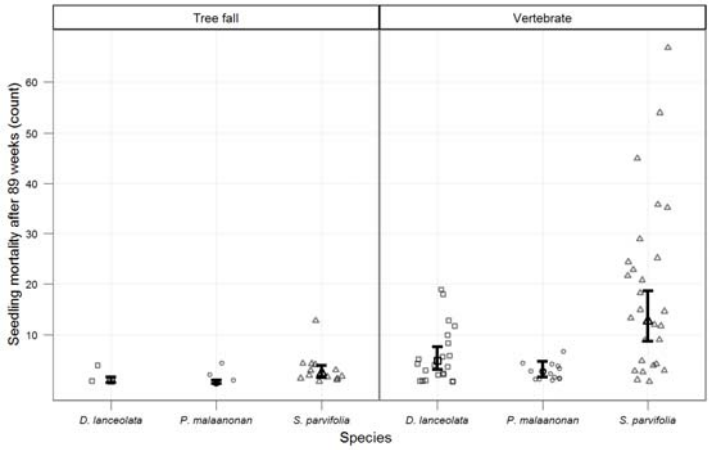

Fig 4 The total counts of seedling mortality (±95% CIs) from all 89 weeks of the experiment as a function of observed mechanism and which mature tree species was nearby. Vertebrate mortality was usually due to trampling from pigs or elephants based on tracks through plots. The grey points in the background represent observed plot counts near each of the three mature species, and the black points represent species means (*D. lanceolata*: open squares; *P. malaanonan*: open circles; *S. parvifolia*: open triangles).

Recruitment dynamics

Overall observed survival declined sharply until 34 weeks after the first census (Fig 5). Following the 34th week, seedling survival remained relatively stable with stochastic events such as tree falls and trampling by mammals causing the majority of seedling mortality (Fig 5). Negative density dependence only affected recruitment of con-specific seedlings around *D. lanceolata* which had a slope significantly lower than one (β_{slope} = 0.77, 95% CI: 0.54 – 0.99; Fig 6). The slope of total seeds to hetero-specific seedling recruitment at *D. lanceolata* was steeper than that of con-specific recruitment (β_{slope} = 0.95, 95% CI: 0.64 – 1.28; Fig 6). *P. malaanonan* and *S. parvifolia* con-specific seedling recruitment had slopes which did not differ from one (β_{slope} = 1.1, 95% CI: 0.8 – 1.39 and β_{slope} = 0.86, 95% CI: 0.68 – 1.03 for *P. malaanonan* and *S. parvifolia*, respectively; Fig 6). The slope of total hetero-specific

seeds to seedling recruitment around *S. parvifolia* trees was nearly equal to the slope of con-specific seedlings (β_{slope} = 0.83, 95% CI: 0.62 – 1.02). One hetero-specific seedling recruited near mature *P. malaanonan* trees, and we therefore did not estimate a slope for hetero-specifics near that species (Fig 5 and 6).

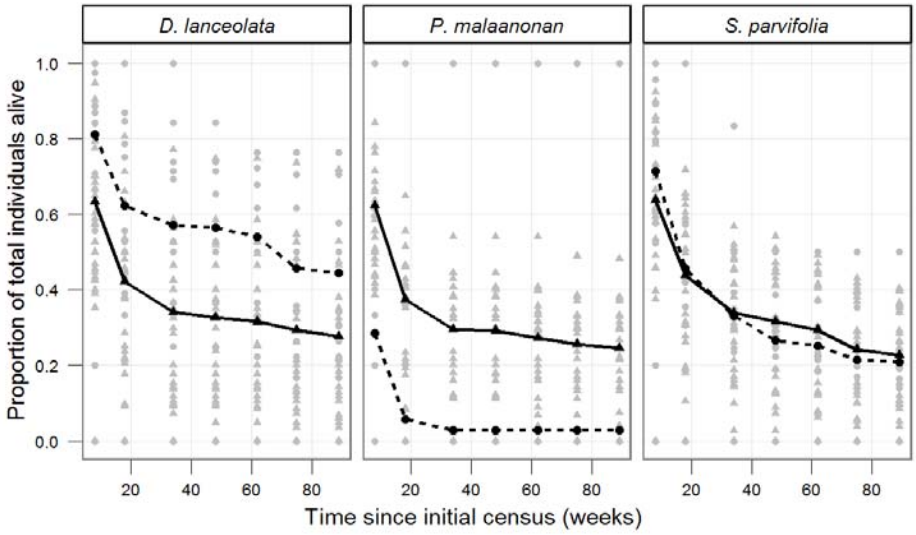

Fig 5 The observed proportion of seedlings surviving to each census point for con-specific seedlings of each mature tree species (triangles and solid lines) and hetero-specific seedlings (circles and dashed lines). The grey points represent the proportion of mortality at each plot at each census. One hetero-specific seedling near *P. malaanonan* mature trees remained alive after the 34th week. Mortality slowed following the 34th week.

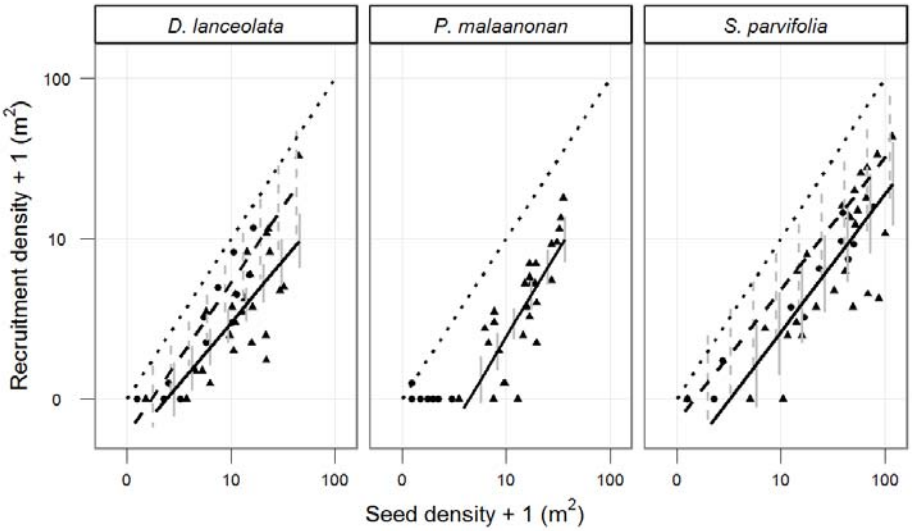

Fig 6 The log-log relationships between total seed fall and seedling recruitment for con-specific seedlings of the mature tree species (triangles and solid lines) and hetero-specific seedlings (circles and dashed lines). The dotted line represents a slope of one; below this slope indicates negative density dependence was acting on seedling recruitment. Only one hetero-specific seedling recruited near *P. malaanonan* and was therefore the log-log relationship was not analyzed. Negative density dependence was found for only con-specific seedlings recruiting near *D. lanceolata*.

Discussion

Our analysis of early regeneration dynamics in a logged forest during a large-scale masting event provides evidence for decreased mortality of rare seeds and reduced mortality of invading hetero-specific seedlings. At the seed stage, greater mortality of con-specific s by local fungi and predation by invertebrates of locally abundant seeds drove these results. However, evidence for negative density dependence at the seedling and recruitment stage as a driving mechanism was limited to reduced hetero-specific mortality compared with con-specific mortality. Density independent mortality such as falling trees and trampling by vertebrates were the major mechanisms of mortality at these two stages. The strength of competitive

dynamics will increase with time, as seedling size increases and competitive exclusion begins. Therefore, in the longer-term a re-evaluation will be necessary to determine whether or not greater intra- versus inter-specific competition among seedlings is a driving force in logged forests.

Seed mortality

Locally rare seeds had an advantage over abundant species (Fig 1). This advantage could represent a preference of the local invertebrate and small vertebrate communities for abundant seeds (Connell 1971). In support of our results, Hautier et al. (2011) found that small mammal predation was higher on con-specific seeds than on transplanted hetero-specifics. We found no indication of greater predation by small mammals with greater seed density probably due to the high seed production throughout the entire region compared to more local events that have been studied (Maycock et al. 2005; Hautier et al. 2010). Additionally, pathogens caused the most con-specific seed mortality. Soil near parent trees may contain higher concentrations of fungi specific to the species and would favor the establishment of hetero-specific species which may be resistant to local fungi in turn promoting seedling diversity (Janzen 1970; Augspurger & Kelly 1984; Packer & Clay 2000; Reynolds et al. 2003; Bell et al. 2006; Freckleton & Lewis 2006).

The large size of the 2010 masting event in terms of both geographic extent and percentage of flowering trees contributed to the high seed density and survival (Kettle et al. 2011; Fig 5 and S1). Previous studies had fewer trees which produced seeds and flowering was often restricted to a local site, which resulted in near complete recruitment failure (Blundell & Peart 2004; Maycock et al. 2005; Bagchi et al. 2011; Hautier et al. 2010). However, compared with a similar scale masting event

(where 88% of adult trees were estimated to have flowered; Curran & Leighton 2000), we found double the proportion of surviving seedlings 34 weeks after seed fall (see Fig 7 in Curran & Webb 2000). This high survival was likely due to predator satiation either from extremely high seed densities during this event or a reduced predator population in the logged forest. In conjunction, generalist vertebrates may have concentrated in areas with higher densities of mature dipterocarps relative to the more isolated individuals used for this study. The former seems more likely as bearded pig activity was quite high in the surrounding forests and evidence for seed predation by bearded pigs was found in most plots (Fig 2; O'Brien, *personal observation*).

Seedling mortality

The probability of seedling mortality increased with density for all species (Fig 3), although for *S. parvifolia* the relationship was negative at very low canopy openness (< 2 %). High density patches may both attract generalist vertebrates to an area or encourage vertebrates to remain in productive areas (Curran & Webb 2000). Increased intra- versus inter-specific competition may account for the mortality difference between con- and hetero-specific seedlings, although there was no direct evidence for this in our dataset. Con-specific seedlings commonly had higher densities in plots and may have already begun to compete for growing space. *D. lanceolata*, which quickly grows to larger heights than the other two species, may have already begun intra-specific competition which would explain the negative density dependence for that species (Fig 6). Conversely, *S. parvifolia*, which grows slowly under low light and quickly in high light (Philipson et al. 2012), only had a positive relationship between mortality and density under open canopies. The smaller *S. parvifolia* seedlings under closed canopies had not begun competing whereas larger

seedlings under open canopies were already competing. This interaction may indicate that competition between seedlings occurred at a density-size relationship that had been reached in open canopy plots but not yet in closed canopy plots.

The probability of hetero-specific seedling mortality was lower than con-specific seedling mortality around mature *D. lanceolata* and *S. parvifolia* trees. The majority of seedling mortality occurred between week 8 and week 34 following which it leveled off (Fig 5). Seedlings were most vulnerable at the early stage when they were dependent upon cotyledon reserves and limited leaf area for defense from and recovery to pathogens and herbivores (Fenner 1987; Paine et al. 2009). Local vertebrate and invertebrate communities may preferentially attack con-specifics, which would increase their chances of mortality.

The high hetero-specific mortality near *P. malaanonan* was likely an indirect effect of very few hetero-specific seeds invading near *P. malaanonan*. Therefore, a few seedling deaths equaled a high probability of mortality as only a few individuals invaded. Only one mature hetero-specific tree was found within 20 m of a *P. malaanonan* and it was both down-wind and down-slope. *D. lanceolata* and *S. parvifolia* also only had one hetero-specific mature dipterocarp within 20 m but in both cases they were up-wind or up-slope causing increased invasion of seeds. Density estimates of mature dipterocarps in logged forest indicate a mean (SD) of 18 (12) trees per ha^{-1} (Berry et al. 2008). The low density and patchy distribution of mature trees limited hetero-specific seed invasion into the areas surrounding the more isolated mature trees (Turnbull et al. 2000; Paine & Harms 2009). This dispersal limitation could lead to mono-dominant islands around mature trees. These islands could persist until juvenile dipterocarps mature and reduce the average distance between mature dipterocarps allowing increased hetero-specific invasion.

Recruitment

Negative density dependence only affected con-specifics around *D. lanceolata* (Fig 6). This result could indicate that seedlings of the other two species were not utilizing all the growing space due to their smaller size; therefore, competition for resources had not begun (Chesson 2000). Additionally, a large proportion of seedling mortality was driven by density independent mechanisms (Fig 4). The former hypothesis was supported by the positive relationship between mortality and density under more open canopies (Fig 3). Under high light conditions, seedlings grow faster and become large in relatively short time periods (Philipson et al. 2012). Therefore, larger seedlings under canopy gaps were already competing where small seedlings under closed canopies were not.

Conclusion

Our results show that some of the processes that promote diversity in primary tropical forests were operating in the logged forest in this study. In response to our initial questions, 1) seed dispersal between neighbouring trees was observed, 2) locally rare seeds had lower probability of mortality than abundant seeds and 3) hetero-specific seedlings had greater probability of mortality than con-specifics. However, the evidence at this stage cannot predict long-term recruitment to answer the 4th question. Overall the major bottle-neck appears to be the low density and diversity of mature trees which causes dispersal limitation of selectively logged dipterocarps and inhibits the local diversity of regenerating seedlings. The low diversity of seedlings across the landscape in our study supports the concept of enrichment planting as a potential management practice to accelerate forest recovery.

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Supplement

CI and p-value estimation

We calculated the 2.5 and 97.5 percent quantiles from the posterior distribution of the parameter estimates resampled 1000 times in order to estimate 95% confidence intervals (CIs). We estimated *p*-values using the equation from Bagchi et al. (2011) derived from Gelman & Hill (2007). To test whether the slope of total seed fall to recruitment was significantly different from one, we calculated the difference from one of each estimated slope for 1000 resamples of the posterior distribution and used the same equation as above to estimate the *p*-value. To test whether the slopes of con- and heterospecific recruitment were different from each other, we calculated the difference between each estimated slope for 1000 resamples of the posterior distribution and estimated the *p*-value with the same equation.

Dispersal statistics

In order to identify seed dispersal distribution around isolated trees, we analyzed total seed fall at each mature tree species as a function of mature tree species, distance from mature tree and relatedness to mature tree. We used generalized-linear mixed models with a Poisson distribution and a log link function. A random intercept term for each mature tree was used to account for individual variability in seed production. We also analyzed seed fall as a function of canopy openness with the same distribution and error terms in order to ensure density of seeds was not collinear with canopy openness.

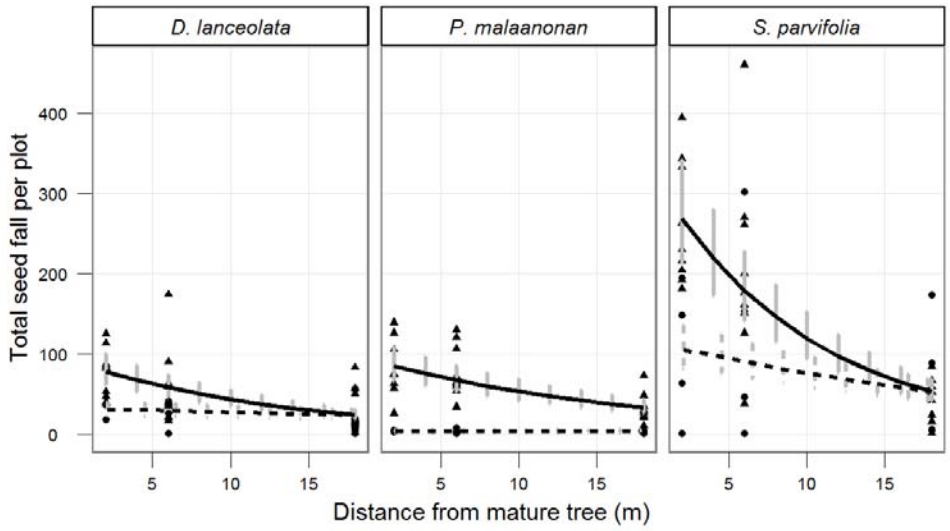

Fig S1 The mean total seed fall density (±95% CIs) as a function of distance from and species of mature tree. Lines represent the fitted model for con-specific (solid lines) and hetero-specific (dashed lines) seeds. Points represent observed total seed fall densities at each plot following the masting (con-specific: triangles and hetero-specific: circles). Con-specific seed fall decreased with distance from mature tree but the rate of decline varied with species ($\beta_{distance}$ = -0.07, 95% CI: -0.08 – -0.06, p < 0.0001, $\beta_{distance}$ = -0.06, 95% CI: -0.08 – -0.03, p < 0.006 and $\beta_{distance}$ = -0.1, 95% CI: -0.12 – -0.08, p < 0.0001, for *D. lanceolata*, *P. malaanonan* and *S. parvifolia*, respectively). Hetero-specific seed fall also decreased with distance from mature tree but had a significantly flatter slope ($\beta_{distance}$ = 0.058, 95% CI: 0.05 – 0.07, p < 0.0001). The smallest seeded species, *S. parvifolia*, had significantly more con-specifics seeds per plot than the other two species ($\beta_{parvifolia}$ = 1.29, 95% CI: 0.93 – 1.64, p < 0.0001). There were significantly fewer hetero-specific seeds near mature trees of *P. malaanonan* ($\beta_{malaanonan*hetero}$ = -2.2, 95% CI: -2.58 – -1.82, p < 0.0001). Canopy openness had a non-significant relationship with density (β_{canopy} = 0.04, 95% CI: -0.02 – 0.09, p = 0.16).

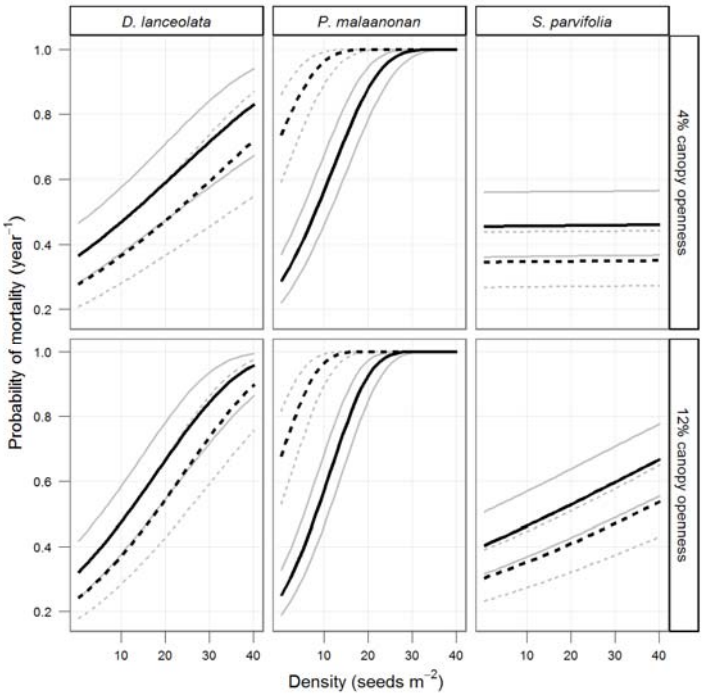

Fig S2 The probability of seedling mortality per year (±95% CIs) as a function of total seedling density for con-specific seedlings of the mature tree species (solid lines) and hetero-specific seedlings (dashed lines) calculated at a 4 and 12% canopy openness. A significant interaction between density and canopy openness existed such that seedling mortality is greater with higher densities under more open canopies. Both *D. lanceolata* and *P. malaanonan* mortality significantly increased with density while *S. parvifolia* only increased with density at high canopy openness.

Chapter 2

Variable rainfall frequency influences regeneration dynamics in an aseasonal tropical forest

Michael J. O'Brien, John Tay & Andy Hector

Photo courtesy of Roman Kellenberger

Abstract

Climate change induced variation to rainfall patterns will have direct effects on regeneration dynamics of plant species, especially in historically everwet tropical rainforest. Differential species response to infrequent rainfall may influence seed germination and seedling establishment in turn affecting species distributions. We tested the role of watering frequency intervals (from daily to six-day watering) on germination and the early growth of Dipterocarpaceae seedlings in Borneo. We used seeds that ranged in size from 500 to 20000 mg in order to test the role of seed mass in mediating the effects of infrequent watering. With frequent rainfall, germination and seedling development traits bore no relationship to seed mass, but all metrics of seedling growth increased with increasing seed mass. Cumulative germination declined by 39.4% on average for all species when plants were watered at six-day intervals. Days to germination increased by 76.5% on average for all species from daily to six-day intervals, but the increase was significantly greater for small-seeded species. Final height and biomass declined on average in the six-day interval by 15.5% and 30.3%, respectively, but the percentage decrease in final size was greater for large-seeded species. Rooting depth per leaf area also significantly declined with seed mass indicating large-seeded species allocate relatively more biomass for leaf production. This difference in allocation provided an establishment advantage to large-seeded species when water was non-limiting but inhibited their growth under infrequent rainfall. The observed reduction in the growth of large-seeded species under infrequent rainfall would likely restrict their establishment in drier microsites associated with coarse sandy soils and ridge tops. In total, these species differences in germination and initial seedling growth indicates a possible niche axis that may help explain both current species distributions and future responses to climate change.

Keywords

Borneo, Determinants of plant community diversity and structure, Dipterocarpaceae, Drought, Plant–climate interactions, Sabah Biodiversity Experiment, Seedling establishment

Introduction

Germination and early seedling growth are highly susceptible to changes in climatic conditions such as temperature and water availability (Leishman and Westoby 1994; Dalling and Hubbell 2002; Daws et al. 2002; Daws et al. 2008). In aseasonal tropical forests, species have evolved under everwet conditions which may make these systems especially sensitive to changes in the rainfall regime (Gibbons and Newbery 2003; Potts 2003). Previous research on the effects of drought in tropical forests has mainly focused on total water deficits during long periods of no rain associated with a dry season (Condit et al. 1995; Poorter and Hayashida-Oliver 2000; Engelbrecht and Kursar 2003; Beier et al. 2012). However, in moist tropical forests, the timing and variability in rainfall may have detrimental effects on regeneration regardless of total monthly rainfall (Blaine and Kellman 1991; Engelbrecht et al. 2006).

In Borneo, rainfall varies greatly on daily, weekly and monthly timescales despite it being classified as a moist aseasonal climate (Walsh and Newbery 1999). Additionally, the forests of Borneo have evolved with El Niño Southern Oscillation (ENSO) events which are associated with drier conditions and more variable rainfall, defined as short-term rainless periods followed by extreme rainfall (Curran et al. 1999; Walsh and Newbery 1999). Seedling establishment in these forests may be especially susceptible to infrequent rainfall because Dipterocarpaceae (dipterocarps)

which dominate the primary forest canopy often have recalcitrant seeds (i.e. short viability with no soil seed banks due to desiccation sensitivity), and their seedling recruitment relies on the episodic mast fruitings, which commonly coincide with ENSO events (Ashton et al. 1988; Curran et al. 1999). Furthermore, the effect of rainfall variability on plant growth is increasingly relevant as global precipitation cycles are expected to intensify with climate change (Huntington 2006; Heisler-White et al. 2009; Beier et al. 2012). In the tropics, an increase in both frequency and intensity of ENSO events could have substantial impacts on tropical forest dynamics, especially at the sensitive seed and seedling stage (Foster 1986; Fenner 1987; Timmermann et al. 1999; Walsh and Newbery 1999; Engelbrecht et al. 2006; Huntington 2006; Lewis et al. 2011).

Seed size is an important trait which determines species success to climatic stress (Leishman and Westoby 1994; Muller-Landau 2010). Larger seeds often have deeper root extension beyond the drying soil profile and greater sugar reserves which provides a competitive advantage under stressful drought conditions ((Baker 1972; Leishman and Westoby 1994; Westoby et al. 1996; Dalling and Hubbell 2002; Daws et al. 2007; Muller-Landau 2010). For example, Daws et al. (2008) demonstrated that larger-seeded species were able to germinate at lower water potentials, implying tolerance to drought-imposed desiccation. The success of seedling establishment under infrequent rainfall may be directly related to seed mass (Muller-Landau 2010).

We germinated seeds under multiple different frequencies of water availability to examine the effects of rainfall variability on early seedling establishment. We used species spanning a range of more than two orders of magnitude in seed size to assess the importance of seed mass in mediating the potential negative impacts of infrequent

rainfall. We examined the role of seed mass in seed germination and seedling growth by measuring both pre- and post- germination response.

Methods

Study site

The experiment was conducted between 4 August and 8 November 2010 at the Sabah Biodiversity Experiment (SBE; N05º05'20'' E117º38'32''; 102 MASL). This site is located ≈22 km north of Danum Valley Research Center (DVRC) in the state of Sabah, Malaysia (Hector et al. 2011). Mean annual rainfall (SE) from 1986-2010 from DVRC was 2848.5 (94.0) mm. This experiment was conducted *ex situ* under two layers of 70% shadecloth and thin transparent polyethylene sheeting to exclude rainfall. The shadehouses at the SBE are raised with grated flooring, excluding large mammals but not small rodents and insects. The daily mean temperature (SE) under the polyethylene sheeting during the course of the experiment was 25.3° C (0.08) with a minimum of 21.5° C and a maximum of 34° C. The mean (SE) percent direct light was 4.7% (0.1) and a red:far-red ratio of 1.11 (0.01) (measured by simultaneous shadehouse and open sky photosynthetically active radiation sensors (SKP 210 quantum sensor; Skye instruments LTD, Llandrindod Wells, Powys, UK)) which created a light environment similar to a small gap in the forest understory. A nearby forest gap (approximately 8 m^2) in the Malua Forest Reserve had 4.98% (0.002) direct light and a red:far-red ratio of 1.04 (0.002).

Seed collection

Seeds of eight dipterocarps were collected from the Malua Forest Reserve surrounding the SBE during the masting event which began in late July 2010. Species

were selected to ensure a variety of genera, seed size and morphological characteristics (Table 1). Visibly healthy seeds (i.e. free of fungus, decay and herbivore damage) were checked for maturity by opening five to ten seeds per species and ensuring that the radicle was fully formed. Seed wings were removed; seeds were checked for viability in water (i.e. seeds that sank in a basin of water were deemed viable); and individual seed mass recorded.

An additional 50 seeds of every species were used to generate estimates of seed dry biomass. These seeds were weighed, dried at 64° C to a constant weight and reweighed. Regressions were developed for each species relating dry and wet mass.

Watering frequency and planting

Pots were filled with homogenized forest soil obtained from the Innoprise-FACE Foundation Rainforest Rehabilitation Project (http://www.face-thefuture.com) (pot sizes: 7.0 x 23.0 cm for large-seeded *S. macrophylla* to allow greater soil volume; 4.5 x 22.0 cm for all other species). The soil used was classified as a clay comprised of 50% clay, 30% silt and 20% sand similar to upslope sites of the Malua Forest Reserve (O'Brien *unpublished data*). Total monthly rainfall (30 day period) was set at 240 mm and dispensed at four frequencies to assess the role of rainfall pattern on germination and seedling development.

The water quantity was calculated based on the area of the pots and the millimeters of water per day for each watering frequency. This quantity equated to approximately 13, 25, 51 and 63 ml pot^{-1} per watering for daily, two, four, and six-day frequencies. This watering regime altered frequency while sustaining an equal monthly rainfall in order to test the effects of frequency by removing water deficit. Watering frequencies were chosen based on analysis of monthly rainfall records at the

Table 1 Summary table of Bornean climax species and seed traits ordered from largest to smallest seed mass.

Species (Acronym)	Wet mass (mg) Mean (95% CI)	Dry mass (mg) Mean (95% CI)	Seed description[a]	Days to germination[a]
Shorea macrophylla Ashton (SM)	55229 (54606-55853)	21248 (20996-21500)	thick woody seed coat, recalcitrant	14-37
Parashorea tomentella Meijer (PT)	6533 (5910-7157)	4228 (3976-4480)	thick woody seed coat, recalcitrant	14-184
Dryobalanops lanceolata Burck (DL)	6483 (6042-6924)	2714 (2536-2892)	green soft seed coat, recalcitrant	7-13
Parashorea malaanonan Merr. (PM)	3840 (3217-4464)	1760 (1508-2012)	thick woody seed coat, recalcitrant	14-184
Hopea nervosa King (HN)	2909 (2285-3533)	1272 (1020-1523)	thin hard seed coat, recalcitrant	8-48
Shorea beccariana Burck (SB)	2756 (2133-3380)	944 (742-1245)	thin hard seed coat, recalcitrant	Unknown
Shorea parvifolia Dyer (SP)	853 (230-1477)	514 (262-766)	thin hard seed coat, recalcitrant	7-52
Shorea argentifolia Symington (SA)	809 (230-1477)	485 (233-737)	thin hard seed coat, recalcitrant	8-15

[a] Descriptions and germination range taken from Ng 1992 and recalcitrance taken from KEW database.

DVRC. The daily frequency was based on the maximum number of days with rain in a 30 day period and six-day frequency was based on the minimum number of days with rain in a 30 day period from historical rainfall records (5 days in April 1998; Walsh and Newbery 1999). The two and four-day frequencies provided approximately the mean and 25% quantile of rainfall days. One hundred seeds of each species were placed in pots and randomly assigned to one of the four treatments (25 seeds per species per treatment). Seeds were placed on their side on the soil surface to replicate natural seed position in the forest and allow for monitoring of germination. All pots were watered to saturation prior to planting (Fig. 1).

Soil matric potential

Pots were measured for volumetric soil with an ML2x Theta Probe and HH2 moisture meter (Delta-T Devices, Burwell, Cambridge, UK) from day zero to eighty-four. Measurements were taken before and one hour after watering to determine minimum water content and the extent to which the soil was rehydrated. To calculate a drying curve for the clay soil used in the experiment, the relationship between soil matric potential (ψ) and volumetric soil moisture was determined using the filter paper method (Deka et al. 1995). A range of volumetric soil moisture from1.5% to 46% was used to develop two linear equations between volumetric soil moisture and ψ. Two equations were required because ψ declined at a faster rate below 28% volumetric soil moisture (Fig S1).

Seed and seedling monitoring

Seeds were monitored daily for germination (radicle emergence) and mortality. Only seeds with visible signs of mortality were recorded (i.e. fungus, mammal browse, insect browse, or desiccation). Following germination, seedlings

were monitored daily for mortality and leaf formation. All surviving seedlings were harvested on their 60th day. Roots were extracted and measured for length. Leaf photographs were taken for calculation of leaf area. Seedlings were dried to a constant temperature at 64° C, and leaves, stem and roots weighed.

Analysis

The effect of watering frequency, pre- and post-watering measurements, species, day and their interaction on ψ was analyzed with a general least squares (gls; allowing for non-constant variance of different species) in the nlme package for R version 2.13.2 (Pinheiro and Bates 2000; R Development Core Team 2011). Including a factor for species identity never improved the model fit, so it was dropped from the soil matric potential analysis.

Phenological and morphological traits (cumulative germination; number of days to germination; number of days to leaf formation; diameter (mm); height (mm); root length (mm); total dry biomass (g); leaf area (cm^2)) in the daily watering were compared using linear mixed effects models (with species identity as a random effect) to assess differences in baseline performance across the seed mass spectrum. Seed mass was $\log_{10}$ transformed for all analysis to meet assumptions of linearity.

The effect of water frequency on seedling traits was assessed by pooling traits of all species in each watering frequency. Species variance from the pooled mean was accounted for with a random effect of species varying within treatment. The lmer function in the lme4 package was used for this analysis due to the added complexity of the crossed random effects.

In order to examine the tolerance to infrequent watering, the mean relative difference between daily and six-day watering of each species was calculated for cumulative germination, days to germination, final total biomass and height (eq. 1).

$$stress\ response = \frac{\bar{y}_{i,six} - \bar{y}_{i,daily}}{\bar{y}_{i,daily}} \quad \text{eq. 1}$$

Where $\bar{y}_{i,six}$ is the mean performance of trait *i* in the six-day watering frequency, and $\bar{y}_{i,daily}$ is the mean performance of trait *i* in the daily frequency. Stress response equates the effect of infrequent watering for a given trait in proportion to its baseline performance (Engelbrecht et al. 2006). Large differences from zero can be interpreted as a trait which was sensitive to stress caused by infrequent watering where small differences would be a trait tolerant to infrequent watering. Linear models compared these proportions with species seed mass to assess the relationship between seed mass and stress tolerance for germinating seeds and early seedling growth. We examined the change in biomass allocation metrics (i.e. shoot:root (g g^{-1}; SRR), root mass:total mass (g g^{-1}; RMR) and root depth:leaf area (mm cm^{-2}; RLA) ratios) with seed mass to explain trends in stress response using the same linear methods used to assess baseline trait differences.

Results

Soil matric potential

Pre-watering ψ was significantly lower at the end of each four and six-day watering frequency but recovered to that of daily and two-day after watering (Fig. 1; Table S1 [significant pre-watering*treatment interaction]). The difference between pre- and post-watering ψ increased throughout the course of the experiment and at different rates for each treatment (Fig. 1; Table S1 [significant three-way

interaction]). Initial mean (SE) pre-watering ψ for all watering frequencies was approximately -4 kPa (Fig. 1). At the final measure on the 84th day, mean (SE) pre-watering ψ for daily, two, four and six-day watering was -10.2 (0.7), -19.6 (4.6), -93.1 (30.1) and -154.4 (54.6) kPa, respectively (Fig. 1).

Baseline performance

We found large species-specific differences in phenological and morphological traits (Fig. S2) with growth traits clearly related to seed size. Days to germination and leaf out had no relationship with seed mass (Fig. S2), but cumulative germination declined with seed mass by -8 seeds (95% CI:-13 – -3; Fig. 2) per $\log_{10}$ mg. The particularly low germination of *S. macrophylla* drove the relationship between cumulative germination and seed mass. If *S. macrophylla* was removed, then the slope between cumulative germination and seed mass did not differ significantly differ from zero (slope = -3, 95% CI:-10 – 3). Height, diameter, root, biomass and leaf area increased with seed mass by 73.5 mm (95% CI: 15.3 – 131.7), 18.6 mm (95% CI: 12.6 – 24.7), 25.0 mm (95% CI: 3.5 – 46.5), 0.74 g (95% CI: 0.47 – 1.00) and 135.8 cm^2 (95% CI: 87.1 – 184.7) per $\log_{10}$ mg, respectively (Fig. 2; Fig. S2). The largest seeded species *S. macrophylla* (Fig. 2; open circle) had the lowest number of seeds germinate but had higher biomass, diameter, height and leaf area growth after 60 days of growth (Fig. S2).

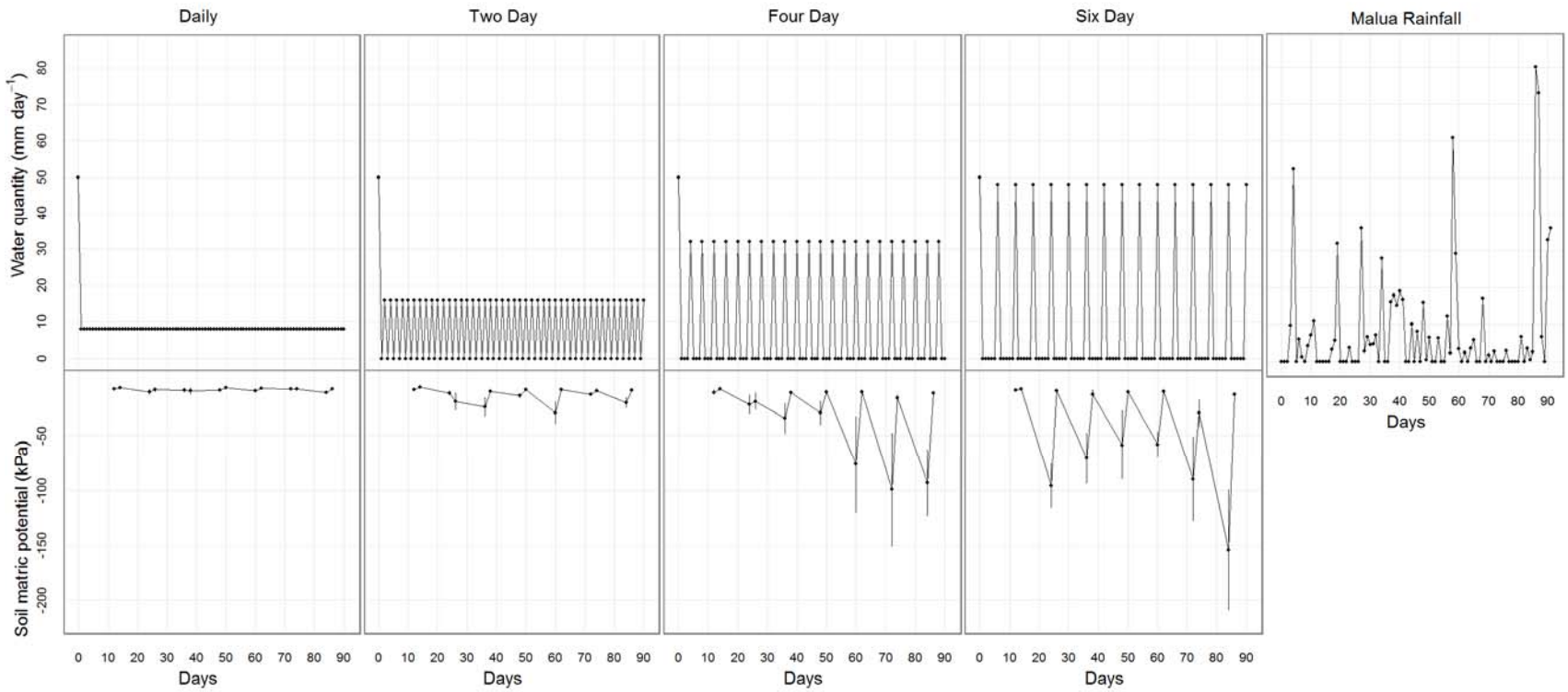

Fig. 1 The frequency of watering per treatment as a function of time, and the rainfall recorded at the SBE over the 90 days of the experiment (far right panel). Each treatment received 720 mm of water and actual rainfall was approximately 724 mm over the course of 90 days with 44 near rainless days (<1 mm day^{-1}). The lower panels show the change in soil matric potential (ψ) with each treatment (pre- and post-watering measures are jittered for readability)

Water frequency, species traits and seed mass

Altering water frequency from daily to six-day watering negatively affected most traits. Mean (daily – six-day) cumulative germination (20.9 – 13.1 seeds), height (158.4 – 118.4 mm), diameter (30.8 – 19.3 mm), root depth (93.7 – 83.1 mm), biomass (1.1 – 0.4 g) and leaf area (92.3 – 38.4 cm^2) declined with infrequent watering while days to germination (9 – 15 days) increased from daily to six-day watering, respectively (Fig. 3; Fig. S3). Timing of leaf formation and root depth per leaf area were not affected by infrequent watering (Fig. S3).

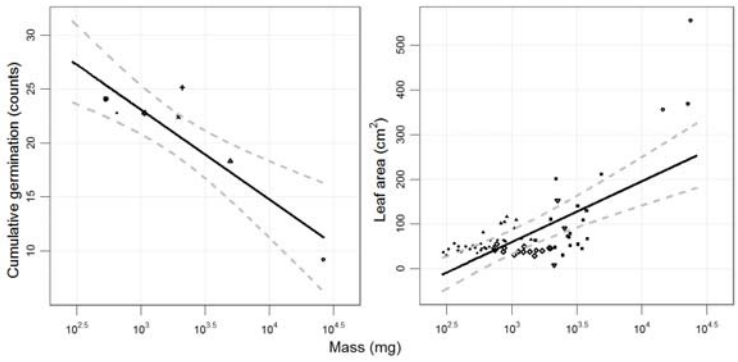

Fig. 2 The relationship of baseline cumulative germination and seedling leaf area as a function of seed mass in the daily watering treatment (mean ± 95% CI). Leaf area was based on the last harvest after 60 days. Points represent individual seedlings. (SM: open circle, PT: upward triangle, DL: closed square, PM: downward triangle, HN: open diamond, SB: closed triangle, SP: closed diamond, SA: closed circle)

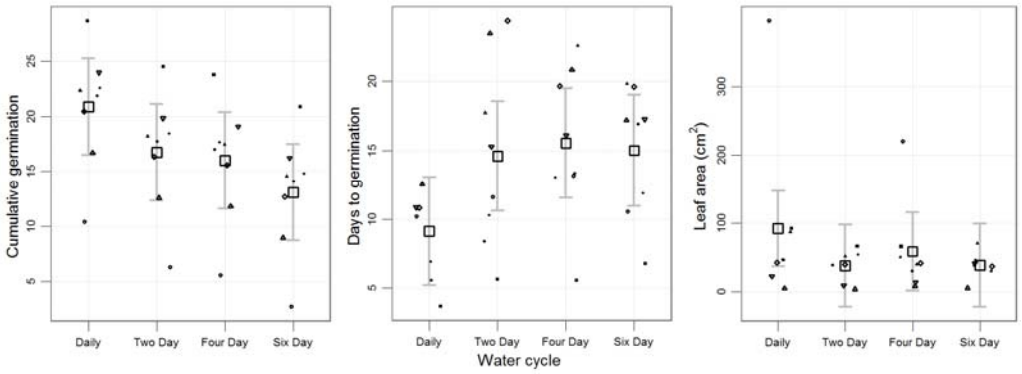

Fig. 3 Cumulative germination, days to germination and final seedling leaf area calculated as a function of treatment. Leaf area was calculated from the last harvest after 60 days. The large open squares represent the pooled mean (± 95% CI) of the eight Bornean climax species. All three traits were negatively affected by infrequent watering. Individual species points represent their estimated random distribution around the mean from the model. The points are jittered for readability. (SM: open circle, PT: upward triangle, DL: closed square, PM: downward triangle, HN: open diamond, SB: closed triangle, SP: closed diamond, SA: closed circle)

There was no relationship between stress response and seed mass for cumulative germination (Fig. 4). However, seed mass was significantly related to stress response for days to germination ($F_{1,6}$ = 54.5, p = 0.0003), height ($F_{1,6}$ = 38.7, p = 0.0008) and total biomass ($F_{1,6}$ = 63.7, p = 0.0002) (Fig. 4). Days to germination increased by as much as 217% for *S. argentifolia* (the smallest seeded species) and as little as a 10.4% decrease for *S. macrophylla* (i.e. no effect for the largest seeded species) from daily to six-day frequency. On average (SE) a 76.5% (6.5%) increase in days to germination was observed. The increase in days to germination declined with seed mass (slope = -0.79, 95% CI: 0.53 – 1.06). Height decreased by as much as 36.9% for *P. tomentella* (for the largest seeded species surviving to 60 days) and 8.5% for *S. argentifolia* (the smallest seeded species) from daily to six-day frequency. Height decreased on average (SE) by 25.7% (3.2%) for all species. Large-seeded species had greater differences in biomass between treatments (slope = -0.55, 95% CI:

-0.78 – -0.34). Biomass decreased by as much as 71.4% for *P. tomentella* and 7.9% for *S. parvifolia*, and on average (SE) decreased by 30.3% (8.7%) for all species. Large-seeded species had greater differences in height between treatments (slope = -0.58, 95% CI: -0.76 – -0.41). Not one seedling from the largest seeded species, *S. macrophylla*, survived to the end of the experiment. RLA declined with seed mass by -1.1 (95% CI: -1.55 – -0.55) per $\log_{10}$ mg, but none of the alternative allocation measures showed a significant trend with seed mass (Fig. 5; Fig. S2).

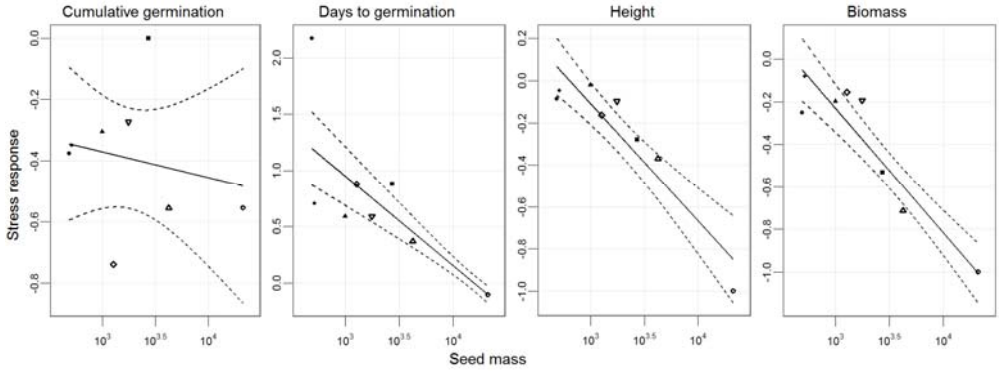

Fig. 4 Stress response (i.e. the proportional difference between daily and six-day watering) as a function of seed mass for cumulative germination, days to germination and seedling height and biomass (mean ± 95% CI). No relationship existed between seed mass and cumulative germination. Germination of large seeds was more resistant to infrequent watering, but post germination large-seeded species had significantly greater declines in growth due to infrequent watering. Points represent species values for stress response. (SM: open circle, PT: upward triangle, DL: closed square, PM: downward triangle, HN: open diamond, SB: closed triangle, SP: closed diamond, SA: closed circle)

Discussion

This study examined the role of infrequent rainfall on seedling establishment of Bornean dipterocarp species focusing on seed mass as a mediating trait. We found that variation in rainfall frequency altered the competitive balance between species

across a seed mass spectrum, with infrequent rainfall favoring large-seeded species before germination but favoring small-seeded species after germination. Under the high rainfall frequency characterizing the aseasonal tropics, all seed sizes took a similar number of days to germinate, and large-seeded species grew to a larger size than small-seeded species. However, infrequent rainfall disproportionately hindered large-seeded species growth compared with that of small-seeded species, which equalized final seedling size between large and small seeded species.

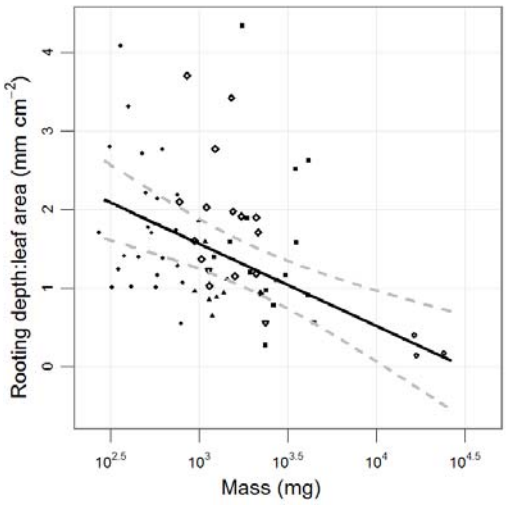

Fig. 5 Root depth per leaf area (mean ± 95% CI) as a function of seed mass. Large-seeded species produce much larger leaves increasing transpiration and water demand without proportionally larger rooting depth leaving them susceptible to breaks in water conductivity and water stress. Points represent individual seedlings. (SM: open circle, PT: upward triangle, DL: closed square, PM: downward triangle, HN: open diamond, SB: closed triangle, SP: closed diamond, SA: closed circle)

Germination

Days to germination showed no simple trends with seed mass suggesting other unmeasured seed traits were likely influential in germination. Seed coat thickness and toughness were two unmeasured traits that likely affected germination. The five

species with the longest days to germination regardless of seed mass had the thickest and most lignified seed coats (Ng 1992; O'Brien, *personal observation*; Table 1; Fig. 2). Alternatively, the species with the fewest days to germination (*D. lanceolata*) was the third largest seed but had a soft, green coat. Thick lignified seed coats would require a softening or breaking for the radicle to emerge. This would result from abiotic forces (e.g. the wetting and drying of the seed coat from water or diel temperature fluctuation) or biotic traits (e.g. increased emergence force from larger radicles associated with larger seeds) (Vasquez-Yanez and Orozco-Segovia 1993; Pearson et al. 2002; Daws et al. 2007). Species may also vary in response to environmental factors such as light quantity and quality and temperature that may account for unexplained variation in days to germination (Daws et al. 2002). Therefore, the timing of germination is driven by complex interactions between seed traits and microsite conditions, which did not simply correlate with seed mass for dipterocarps (Vasquez-Yanez and Orozco-Segovia 1993; Daws et al. 2002).

Infrequent rainfall inhibited days to germination for small-seeded species while large-seeded species remained unaffected (Fig. 4). This temporal germination advantage favors large seeds as they reached greater height and rooting depths which could potentially inhibit establishment and growth of small-seeded species (Muller-Landau 2010). This large seed advantage would be more pronounced under two scenarios. First, under canopy gaps, lower humidity and higher daytime temperatures would increase seed and soil surface drying associated with infrequent rainfall, possibly slowing small seed germination to a greater extent (Poorter and Hayashida-Oliver 2000; Dalling and Hubbell 2002; Daws et al. 2002; Pearson et al. 2002; Engelbrecht et al. 2006). Second, current climate projections suggest that rainfall variability will increase. Walsh and Newbery (1999) have shown that consecutive

months with short-term drought have been more frequent since the last decades of the 20th century. For example, from September 1997 to May 1998 there were four rainless periods of at least 10 days and again from February 2010 to April 2010 there were three rainless periods of at least 7 days (Walsh and Newbery 1999; O'Brien *unpublished data*). Under more frequent or longer rainless periods, large-seeded species would have a germination advantage (Timmermann et al. 1999; Walsh and Newbery 1999). However, dipterocarp seeds likely have a threshold to infrequent rainfall that, if reached during extreme ENSO events, may cause total regeneration failure, especially since many dipterocarps are desiccation sensitive (Ng 1992; Curran et al. 1999).

Cumulative germination declined with seed mass. However, this relationship was driven by high seed mortality, due to a pathogen, of the largest seeded *S. macrophylla*. This high mortality was likely an artifact of the experiment (e.g. the pre-planting treatment or the soil used in this experiment infected the seeds) because other *S. macrophylla* seeds collected at the same time did not experience high mortality from pathogens. This result would need to be retested using a different soil type to ensure cumulative germination declined with increasing seed mass.

There was a reduction in cumulative germination due to infrequent rainfall but it was not related to seed mass (Fig. 4). Natural rainfall patterns during the course of this experiment showed days of high rain and more than ten periods with consecutive no rain days, six of which were > 2 days long (Fig. 1). Infrequent rainfall could reduce overall seed germination on average (SE) by 39.4% (7.9%) which would have marginal impacts on seedling establishment under such ubiquitous seed production. However, greater declines should be expected under projected climate scenarios

where extended dry periods (>10 days) are becoming more frequent (Walsh and Newbery 1999; Engelbrecht et al. 2006).

Seedling growth

Large-seeded species had greater growth under frequent rainfall (Fig. 2). The greater stored sugar reserves often associated with larger seeds allows increased biomass growth and greater absolute height growth before leaf development (Leishman and Westoby 1994; Westoby et al. 1996; Nakagawa and Nakashizuka 2004). Although absolute growth was greater for large-seeded species after 60 days, small-seeded species commonly have higher relative growth rate and may overtake large-seeded species given longer time periods (Baraloto et al. 2005; Philipson et al. 2012).

All growth metrics showed a decline with infrequent watering. The magnitude of the effect was dependent on the metric with total biomass and leaf area having the greatest decline and root depth having the lowest. The greater reduction in above-ground growth compared with below-ground growth at the six-day cycle may indicate greater carbon allocation to access deeper water reserves (mean reduction for all species of 30.0 mm and 10.6 mm for height and root growth, respectively). The overall decline in growth was likely due to a water limitation which inhibited cell expansion and division preventing biomass accumulation (Hsiao 1973). Daily and two-day watering maintained ψ at a constant level throughout the experiment while four and six-day watering caused levels more than ten times lower than daily watering. Soil drying also increased throughout the course of the experiment (Fig. 1) because seedlings began establishing. As root systems depleted soil water to meet growth and transpiration demands, drought stress increased, and growth was limited.

Growth of large-seeded species was more inhibited by infrequent watering than growth of small-seeded species (Fig. 4). After 60 days of growth, an average difference of only 24 mm between *D. lanceolata* (a large seeded species) and *S. argentifolia* (the smallest seeded species) was observed under six-day watering versus 76 mm under daily watering. If this trend were to continue with longer rainless periods, it would alter the competitive rank of the species. The effect was even more pronounced for biomass in which the large-seeded *P. tomentella* (0.61 g – 0.18 g) declined to below the mass of the smallest seeded *S. argentifolia* (0.34 g – 0.25 g). Although soil matric potential did not show a significant difference between species, seedlings with greater water uptake could deplete soil water from their immediate soil rhizosphere causing a break in water conductivity and a more intense drought effect (Sperry et al. 2002). Large seedlings with increased water demands for cell expansion and transpiration were affected to a greater extent.

The inverse relationship between rooting depth per leaf area and seed mass (Fig. 5) may explain the increased effect on large-seeded species. In support of this result, Engelbrecht et al. (2006) also found significantly larger rooting depth per leaf area of smaller seeded species. Greater leaf area with proportionally less access to soil water due to shallow rooting increases the risk of desiccation as large transpiration needs would not be met by the rhizosphere water supply (Sperry et al. 2002). For a seedling to sustain the required water conductivity, it must access an increased soil volume (Sperry et al. 2002). Large-seeded species produced greater leaf area which equated to increased transpiration and subsequent water loss. At the early establishment phase, large seedlings would be at a disadvantage under infrequent rainfall especially in rapidly drying sites such as coarse textured soils and large gaps

(Poorter and Hayashida-Oliver 2000; Dalling and Hubbell 2002; Pearson et al. 2002; Sperry et al. 2002).

Conclusions

This study demonstrates differential responses of large- and small-seeded species to watering frequency and temporal distribution of rainless periods. The timing of rainless periods (either before or after germination) altered the competitive advantage between seed sizes. Short-term droughts delayed germination of small-seeded species which gave a temporal advantage to large-seeded species, but at the seedling establishment phase, large seedlings resulting from larger seeds were at risk of desiccation which inhibits growth and possibly leads to increased mortality. Our result has implications for species distributions and supports work done in Lambir Hlls National Park, Sarawak, Malaysia which found an increase in mean seed mass along a soil moisture and fertility gradient with the largest seed masses found on fine clayey soil textures (Katabuchi et al. 2012). Synthesizing the results from Katabuchi et al. (2012) in the context of the results found in this study may indicate that post-germination rainless periods have a greater impact on community composition and species coexistence than pre-germination rainless periods. Current climate change projections of reduced and more variable rainfall will further alter species germination and establishment patterns which would have long-term effects on community composition and species distributions.

Acknowledgements

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staff at the Sabah Biodiversity Experiment, especially site manager Philip Ulok. This research is Sabah Biodiversity Experiment manuscript X.

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Supplement

Table. S1 Summary of significance for parameters explaining soil matric potential (ψ). Species was removed from the model as it was never significant or improved the fit of the model.

Parameter	Num. DF	Denom. DF	F	P
Treat	3	1231	33.66	<0.0001
Pre-watering	1	1231	204.68	<0.0001
Day	1	1231	110.84	<0.0001
Pre-watering*treat	3	1231	11.84	<0.0001
Day*treat	3	1231	7.03	0.0001
Pre-watering*day	1	1231	20.33	<0.0001
Pre-watering*day*treat	3	1231	3.67	0.0120

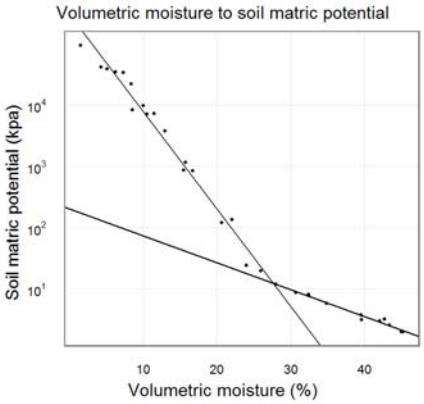

Fig. S1 The curves used to estimate matric potential from volumetric moisture. The matric potential declines at a much faster rate below approximately 28% volumetric moisture (5.477 – 0.1591x; R^2 = 0.977) than above (2.307– 0.044x; R^2 = 0.981).

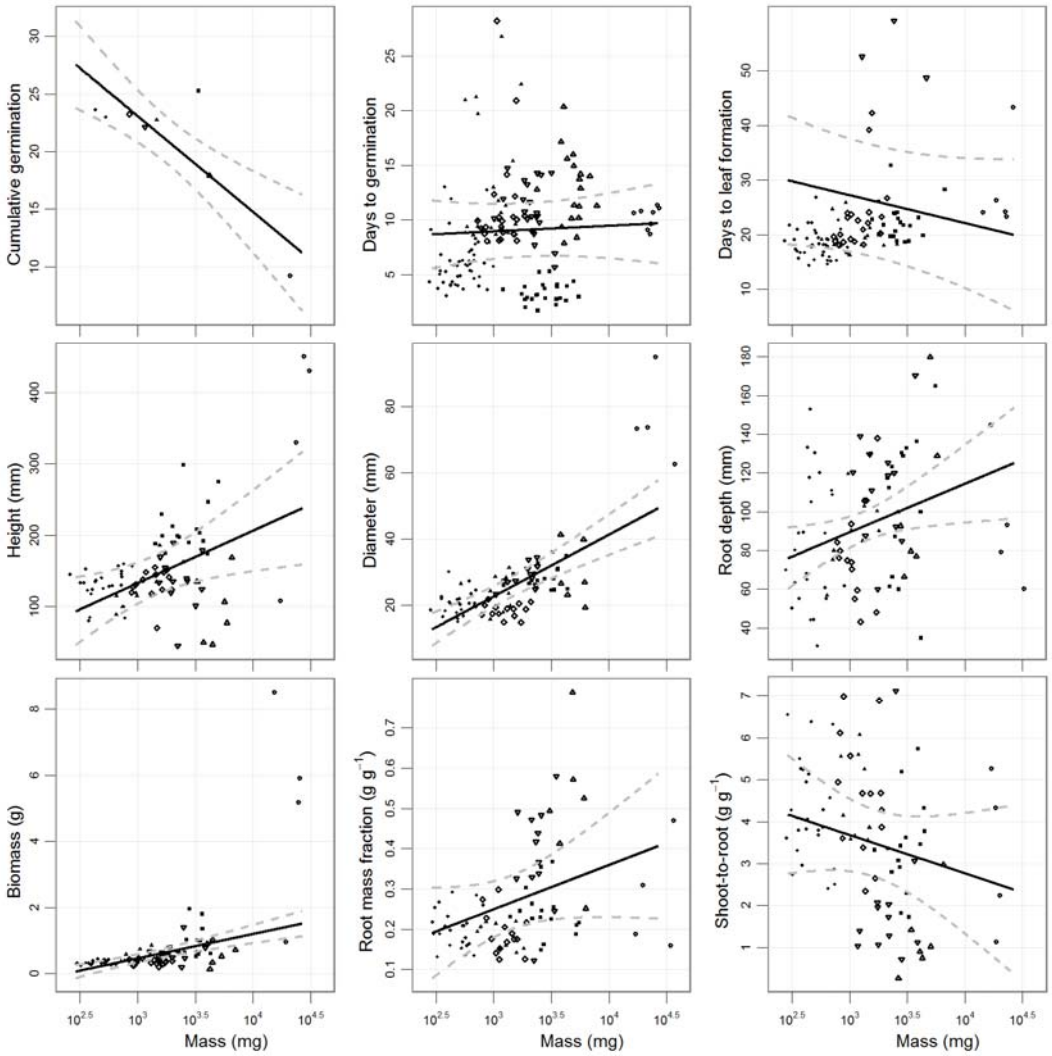

Fig. S2 The relationship of baseline phenological and morphological characteristics with seed mass for germinating seeds and seedlings in the daily watering treatment. Seedling growth and allocation traits were based on the last harvest after 60 days. Solid lines represent model predictions with 95% CIs. Points represent individual observations (SM: open circle, PT: upward triangle, DL: closed square, PM: downward triangle, HN: open diamond, SB: closed triangle, SP: closed diamond, SA: closed circle). Log transforming growth supported untransformed results and was therefore not used for the analysis.

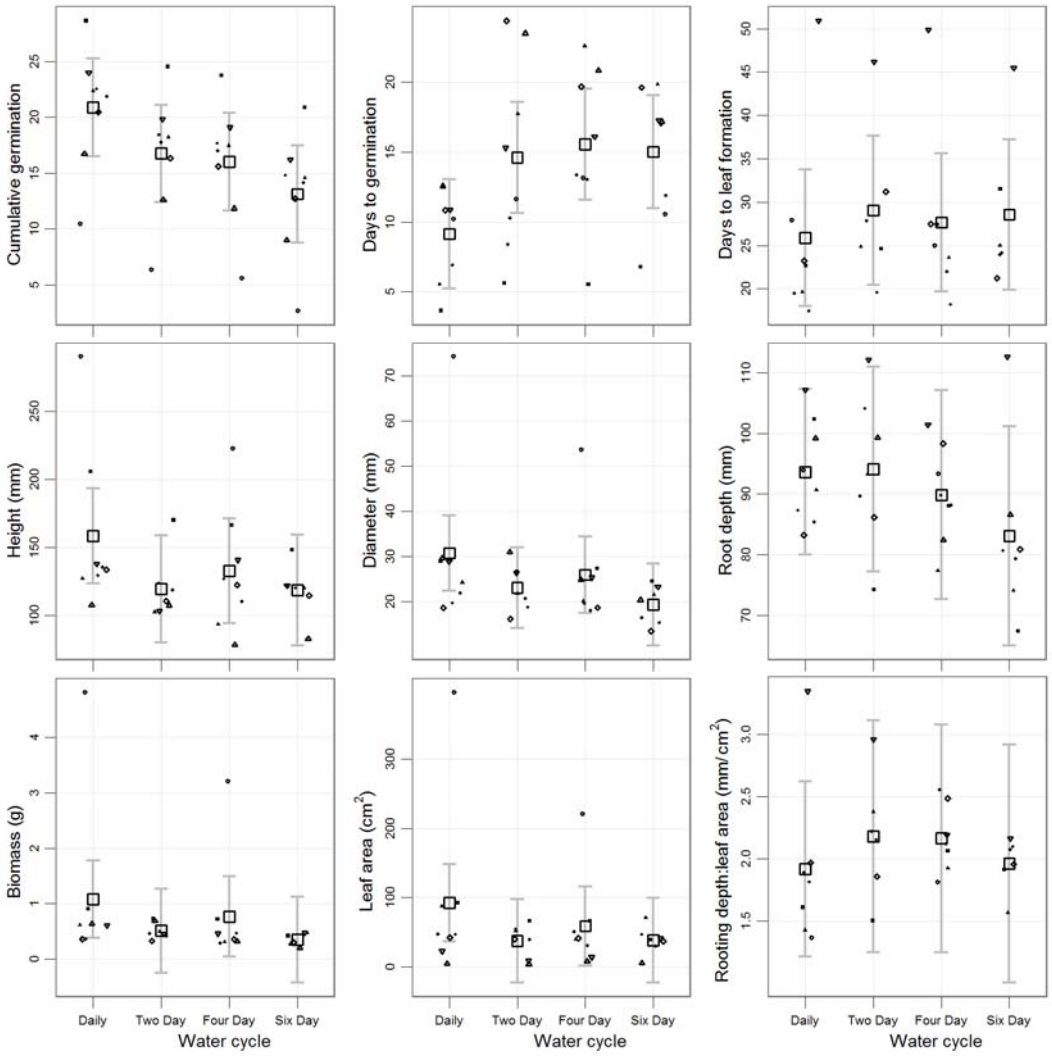

Fig. S3 The effect of water frequency on seed and seedling traits for eight Bornean shade-tolerant species pooled. Seedling traits were based on the last harvest after 60 days. Most traits were negatively affected by infrequent watering. Individual species points were jittered for readability. Open squares represent model predictions with 95% CIs. The smaller points represent random variation of species around the pooled mean for all species. (SM: open circle, PT: upward triangle, DL: closed square, PM: downward triangle, HN: open diamond, SB: closed triangle, SP: closed diamond, SA: closed circle)

Chapter 3

The importance of biomass allocation and physiological response in drought resistance of shade-tolerant seedlings in Borneo

Michael J. O'Brien, John Tay & Andy Hector


Photo courtesy of Roman Kellenberger

Abstract

El Nino Southern Oscillations cause drought at irregular intervals in aseasonal tropical forests. Landscape level mortality has been well studied during these drought periods, but little is known about inter-specific variability in drought tolerance for tree species in many tropical systems, which is important with regard to species distributions. We examined correlations between seedling dieback and biomass allocation, growth and physiological responses for ten Bornean tree species in response to experimental manipulations of two aspects of drought: rainfall variability and deficit. We found that under mild drought stress greater relative allocation to roots limited dieback. However, this response was dependent upon the form of drought with some species more susceptible to rainfall variability and others to deficits. As drought severity increased, species biomass allocation became uncorrelated with dieback, and instead species which sustained high xylem water potentials and stored greater non-structural carbohydrate concentrations had lower dieback. Dieback under severe drought was associated with a growth/storage trade-off in which species with more conservative growth strategies were less susceptible to drought. The large variability in seedling response is consistent with niche partitioning along a drought gradient which has likely influenced landscape level species distributions. Under current climate change scenarios which project more severe and frequent droughts in the aseasonal tropics, future forest composition will be altered through increased mortality of drought sensitive species and increased abundance of drought tolerant species.

Keywords

Borneo; Stomatal conductance; Stem water potential; Species distributions; Non-structural carbohydrates

Introduction

Drought is an increasingly relevant driver in shaping species distributions in tropical forests due to climate change altering rainfall quantity, intensity and frequency (Timmermann et al. 1999; Huntington 2006; Lewis et al. 2011; Beier et al. 2012). Although the response of species to drought has been well studied in seasonal tropical forests, less is known about drought tolerance of species in aseasonal everwet forests which experience irregular droughts associated with El Nino Southern Oscillation events (Walsh and Newbery 1999; Engelbrecht et al. 2003; Engelbrecht et al. 2007; Beier et al. 2012). Previous work in aseasonal forests has largely focused on landscape level mortality rates due to drought (Nakawaga et al. 2000; Gibbons & Newbery 2002; Potts 2003; Slik 2004). However, less has been done to examine the growth and physiological sensitivity of species to drought or the traits relevant to the resistance of species to drought (Burslem et al. 1996; Tyree et al. 1998; Cao 2000; Baltzer et al 2008; Baltzer et al. 2009).

Plant response to drought is often categorized into species that avoid drought and those that tolerate drought (McDowell et al. 2008; Markesteijn & Poorter 2009). Drought avoidance is achieved by reducing transpiration through stomatal closure (Brodribb & Holbrook 2003) and accessing deeper water reserves (Chaves et al. 2002) or under more severe conditions through leaf shedding and aboveground dieback (Brodribb et al. 2003; McDowell et al. 2008; Bartlett et al. 2012). Species that tolerate drought maintain stomatal and hydraulic conductance for continued

growth under dry conditions (McDowell et al. 2008; Bartlett et al. 2012). Additionally, inter-specific differences in above- and belowground biomass allocation are correlated with species sensitivity and mortality to drought (Chaves et al. 2002; Sperry et al. 2002; Engelbrecht et al. 2006; Markesteijn & Poorter 2009). Therefore, variation in species traits such as growth form, biomass allocation and physiological differences (e.g. sensitivity of stomatal conductance and xylem potential to decreasing soil water content) correlate with the strategies of a species and their overall drought resistance.

Drought in many tropical forests is commonly associated with El Nino Southern Oscillations, which can cause extended rainfall deficits as well as more variable rainfall patterns which consist of rainless periods and extreme rainfall events (Burslem et al. 1996; Walsh & Newbery 1999). Highly variable conditions may favor drought 'tolerators' which continue growth through short-term dry periods, while long-term deficits may favor avoiders which survive extended dry periods by maintaining water potentials (Bartlett et al. 2012). These forces may have greater impacts on establishing seedlings than mature trees because they have limited root systems, making them more vulnerable to fluctuations in soil water content (Poorter & Hayashida-Oliver 2000; Engelbrecht et al. 2006). Differential plant response to these aspects of drought (rainfall variability and rainfall deficit) has implications for both current species distributions (Slik et al. 2003; Engelbrecht et al. 2007), and also the recovery of logged forests under an intensifying global water cycle (Chazdon 2003; Huntington 2006).

We examined the sensitivity of seedlings of ten shade-tolerant Bornean tree species to two aspects of drought, infrequent rainfall and severe water deficits. We measured inter-specific variability in growth, allocation and physiological response,

in order to identify key traits for drought resistance. We discuss these results in terms landscape scale species distributions.

Methods

Study site

We conducted this experiment at the Sabah Biodiversity Experiment (N05º05'20'' E117º38'32''; 102 MASL). This site is located approximately 22 km north of Danum Valley Research Center in the Malaysian state of Sabah in north Borneo (Hector et al. 2011). Mean annual rainfall (SE) recorded in Danum Valley from 1986-2010 was 2848.5 (94.0) mm. The experiment was done in 15 shadehouses under two layers of 70% shadecloth. Below the shadecloth clear plastic polyethylene sheeting was used to exclude rainfall. This design provided the seedlings with an average (SE) of 3.9% (0.1) light and a red:far-red ratio (SE) of 1.07 (0.01). Light was measured by simultaneous shadehouse and open sky photosynthetically active radiation sensors (SKP 210 quantum sensor; Skye instruments LTD, Llandrindod Wells, Powys, UK).

The average (SE) daily temperature during the course of the experiment was 25.4 C (0.06) with average maximum and minimum temperature of 30.9 C and 22.7 C, respectively. The average (SE) daily humidity was 92.5% (0.19) with average maximum and minimum humidity of 98.9% and 71.2%, respectively. Temperature within the shadehouses was on average 0.22 C (95% CI: 0.16 – 0.29) hotter but humidity was not significantly higher than values measured in direct light at the Sabah Biodiversity Experiment. Temperature was measured with Thermocron Ibuttons (model DS1921G-F5#, Maxim Integrated Products, Inc., Sunnyvale, CA, USA) at 3 hour intervals throughout the experiment, and humidity was measured

using a relative humidity probe at 30 minute intervals (SKH 2000 probe, Skye instruments LTD, Llandrindod Wells, Powys, UK).

Seedlings

Seeds from ten species of shade-tolerant forest trees were collected during a landscape scale masting event in August 2010 (Table 1). We collected seeds directly from three different mature trees for each species except for *Durio oxleyanus* and *Hopea nervosa* seeds which were obtained from only one mature tree. Seeds were not planted on the same day because seed maturation rates varied among species, but all seeds were planted between the 10th and 29th of August. We weighed each seed and placed them under wet burlap sacks to germinate. After germination, each seed was planted into large pots (20 x 36 cm) within the shadehouses. Pots were filled with homogenized forest soil obtained from the Innoprise-FACE Foundation Rainforest Rehabilitation Project (http://www.face-thefuture.com). The soil used was classified as a clay and comprised 50% clay, 30% silt and 20% sand, which is similar to mid-slope sites at the Malua Forest Reserve. We planted 33-34 seeds from each mature tree (100 for *Durio oxleyanus* and *Hopea nervosa* as there was only one mature tree for each) for a total of 100 seeds per species. Seedlings were watered every two days for three months until all seedlings had dropped their cotyledons. We replanted individuals that died during the first two months from nursery grown seedlings, which were collected from the same mature tree.

Table 1 Species descriptions and their allocation metrics from the frequent watering treatment. Physiological responses of sensitivity of xylem pressure ($d\Psi_x / d\Psi_s$) and stomatal conductance ($dg_s/d\Psi_s$) to decreasing soil water potential were calculated as the slope of that relationship for each species. (Rooting depth:leaf area – RLA; Specific leaf area – SLA; Shoot:root ratio – SRR; Root mass fraction – RMF; Leaf mass fraction – LMF; Leaf area ratio – LAR)

Species (Acronym)	RLA (mm cm^{-2})	SLA (cm^2 g^{-1})	SRR (g g^{-1})	RMF (g g^{-1})	LMF (g g^{-1})	LAR (cm^2 g^{-1})	$d\Psi_x / d\Psi_s$	$dg_s/d\Psi_s$
Dryobalanops lanceolata Burck (DL)	0.69 (0.14)	19.5 (1.1)	3.88 (0.5)	0.22 (0.02)	0.41 (0.02)	81.48 (8.74)	0.6 (1.5)	13.8 (4.0)
Durio oxleyanus Griffith (DO)	0.36 (0.08)	25.65 (1.04)	3.88 (0.5)	0.22 (0.02)	0.37 (0.02)	94.8 (5.20)	4.3 (1.6)	12.0 (3.8)
Hopea nervosa King (HN)	1.31 (0.18)	22.03 (1.22)	3.23 (0.4)	0.25 (0.02)	0.39 (0.04)	87.47 (12.4)	0.04 (0.02)	12.6 (5.7)
Koompassia excelsa Taub. (KE)	0.46 (0.10)	45.66 (2.38)	4.4 (0.5)	0.19 (0.02)	0.45 (0.02)	205.69 (14.3)	0.75 (0.34)	4.5 (3.0)
Parashorea malaanonan Merr. (PM)	1.64 (0.50)	23.27 (2.68)	3.06 (0.5)	0.27 (0.04)	0.44 (0.04)	105.51 (15.4)	0.05 (0.03)	5.1 (4.3)
Parashorea tomentella Meijer (PT)	0.83 (0.18)	21.56 (1.88)	3.43 (0.4)	0.24 (0.02)	0.47 (0.02)	100.98 (10.0)	0.01 (0.02)	18.5 (7.3)
Shorea argentifolia Symington (SA)	1.24 (0.62)	25.56 (1.06)	5.27 (0.7)	0.17 (0.02)	0.54 (0.02)	138.94 (9.5)	0.3 (0.07)	19.8 (4.9)
Shorea beccariana Burck (SB)	1.02 (0.32)	23.24 (1.52)	4.96 (0.7)	0.18 (0.02)	0.56 (0.02)	131.2 (9.5)	0.07 (0.12)	15.2 (4.7)
Shorea macrophylla Ashton (SM)	0.53 (0.32)	14.82 (1.46)	4.57 (0.6)	0.19 (0.02)	0.41 (0.04)	61.84 (8.3)	0.21 (0.16)	15.6 (3.7)
Shorea parvifolia Dyer (SP)	0.94 (0.20)	25.35 (1.62)	5.22 (0.7)	0.17 (0.02)	0.54 (0.02)	137.7 (11.7)	0.5 (0.84)	38.3 (8.2)

Experimental design

On 19 November 2010, we assigned 7-8 seedlings from each mature tree of each species to one of three treatments: 1) 240 mm of water per month distributed every two days (frequent), 2) 50 mm of water per month distributed every 15 days (infrequent) and 3) no water. A total of 22 seedlings per species per treatment were used for the experiment (n = 66 per species).

Seedling monitoring

We monitored the seedlings every two days for changes in leaf morphology. We recorded the date of leaf browning, decline in leaf angle, initial leaf loss and total leaf loss. Every ten days, we made measures of stomatal conductance (g_s) from 08:00 until 18:00 with a steady state diffusion porometer (model SC-1, Decagon Devices, Inc., Pullman, WA, USA). During each daily course, we measured 90 seedlings (three individuals of each species in each treatment) every three hours. Each of the 90 seedlings received three stomatal measurements per daily course (morning, mid-day and afternoon).

Seedling growth, biomass and leaf area

We measured the height, diameter and leaf area of every seedling prior to the start of the treatments and every month thereafter. Leaf area was estimated by measuring the length and width of each leaf on each seedling. These measures were used to make linear models of leaf area from destructive harvests of seedlings. Loss of leaf area from herbivory or dieback was estimated visually as percentage of the whole leaf lost in 5% increments. Leaves were numbered with a water-based permanent marker in order to track individual leaf growth and loss at each measure.

We destructively harvested seedlings prior to the start of the treatments and then approximately 30, 60 and 120 days after the treatments began. A final harvest of all living seedlings occurred 150 days after the treatments began. We harvested one seedling of each species in each treatment at pre-dawn (03:00-06:00), morning (08:00-10:00), mid-day (12:30-15:00) and late-afternoon (17:00-19:00) and made a variety of trait measurements on each individual. In order to examine leaf senescence, we measured chlorophyll content on one leaf from the top, middle and bottom part of the canopy of each seedling using a spadmeter (model Spad-502, Konica Minolta Sensing, Inc., Osaka, Japan). To assess the water status of each seedling, we measured leaf water potential (Ψ_l) on a single leaf (or a cluster of leaves for small seedlings) and stem water potential (Ψ_x) using a Scholander pressure chamber (model 670, PMS Instrument Co., Corvallis, Oregon, USA). We took photographs of all the leaves and analyzed their leaf area using ImageJ software (Rasband 2011). These measured leaf areas were used to generate linear models for estimating leaf area from the length and width measures (Supplement). To assess rooting depth, we removed all soil and measured longest root length. Seedlings were dried at 64 C until a constant weight, and we weighed leaves, stem and roots separately to assess biomass.

Environmental conditions

In order to measure temperature differences among treatments, we placed Thermocron Ibuttons (model DS1921G-F5#, Maxim Integrated Products, Inc., Sunnyvale, CA, USA) in all three treatments in each shadehouse. In order to capture air versus soil temperature differences, Ibuttons were buried 5 cm within the soil and suspended on a stick 5 cm above the soil surface. Volumetric soil moisture content at the top and bottom of the pot was measured on 1-2 seedlings of each species in each treatment every week with an ML2x Theta Probe and HH2 moisture meter (Delta-T

Devices, Burwell, Cambridge, UK). The relationship between soil water potential (Ψ_s) and volumetric soil moisture was determined using the filter paper method (Deka et al. 1995). A range of volumetric soil moisture from 1.5% to 46% was used to develop two linear equations between volumetric soil moisture and Ψ_s. Two equations were required because Ψ_s declined at a faster rate below 28% volumetric soil moisture (O'Brien, unpublished data). We measured temperature and humidity every thirty minutes throughout the course of the experiment, which we used to calculate vapor pressure deficit (Murray 1967; Monteith & Unsworth 1990).

Non-structural carbohydrate analysis

We used three to four seedlings of each species from the first harvest, one seedling of each species in each treatment from all intermediate harvests and three to four seedlings of each species in each treatment from the last harvest to quantify non-structural carbohydrates (NSC) concentrations (percent of biomass) and pools (concentration x organ biomass) in the leaf, stem and root. We ground the organs of each seedling with a ball mill and used 15-16 mg of sample for NSC analysis. We extracted soluble sugars with shaking bath of 80% ethanol at 27 C for one night followed by two additional 2 hour baths (Marquis et al. 1997; Myers & Kitajima 2007). We digested the remaining starch with an amyloglucosidase (A-7420, Sigma-Aldrich, Co., St. Louis, MO, USA). The concentrations of simple sugars and starch were measured at 487 nm by spectrophotometry after a phenol-sulphuric acid reaction (Dubois et al. 1956; Ashwell 1966; Boege 2005).

Analysis

We used linear mixed effects models to assess Ψ_s as a function of treatment, days since the start of the treatment and species, and a random variance component

for shadehouse was used to account for inherent spatial variation in temperature. In order to assess the effect of infrequent watering on changes in Ψ_s, we modeled pre- and post-watering Ψ_s with days since the start of the treatment for the infrequent watering treatment separately. We compared soil temperature among treatments with linear mixed effects models which allowed random variation around hour of the day, individual plant and day.

Total mortality (total count per species) between treatments was analyzed with a linear mixed-effects model using a Poisson variance function. A random effect for species was used to account for inherent species differences. Dieback was defined as leaf area lost as a result of the treatment relative to the frequent watering treatment. We therefore used individual leaf area as a proportion of the average leaf area of the species in the frequent watering treatment at each time point as a metric of dieback. We relativized this value by the proportion of individual leaf area and average leaf area in the frequent watering at day zero (eq 1).

$$\text{dieback} = \ln\left[\frac{area_{ij}}{area_{freqj}} \Big/ \frac{area_{i0}}{area_{freq0}}\right] \qquad \text{eq. 1}$$

Where dieback equals the natural log of the leaf area for an individual in treatment *i* at time point *j* divided by the average leaf area of a species in the frequent watering at time point *j* over the proportion at time point zero (modified from Vojtech et al. 2007). We analyzed the relationship between dieback and days since the start of the treatment for each species in the infrequent and no watering treatments, in order to identify the sensitivity of species to drought. A random effect for individual was used to account for within and among species variation.

In order to identify important traits which confer drought resistance, we used Pearson correlations between mean dieback at multiple time points (60, 90, 120 and 150 days of drought) and mean allocation, growth and physiological traits among them. Xylem sensitivity to decreasing soil moisture potential was calculated as the slope of the linear relationship between mid-day stem and soil water potential ($d\Psi_x$ /$d\Psi_s$; Supplement). Sensitivity of stomatal conductance to decreasing soil moisture was calculated as the slope of the relationship between mean daily stomatal conductance and soil water potential (dg_s /$d\Psi_s$; Supplement). For both of these relationships, random effects accounted for measurement day. Linear growth models of leaf area were used to calculate size-specified growth (SGR; Supplement; Paine et al. 2011) in the frequent watering treatment. We calculated SGR with 125 cm^2 as the specified size, the minimum leaf area achieved by all species before the start of the treatments. We performed a principal components analysis (PCA) of species traits in order to assess how species grouped in two-dimensional space. We removed highly positively correlated traits based on the results of the correlation analysis in order to remove redundancy and simplify interpretation.

All analyses of soil water potential, soil temperature, physiological response and dieback were performed with the lmer function in the lme4 library (Bates & Maechler 2011) of the R statistical software version 2.13.2 (R Development Core Team 2011. Correlations were performed with the rcorr function in the Hmisc library. We calculated the 2.5 and 97.5 percent quantiles from the posterior distribution of the parameter estimates resampled 1000 times in order to estimate 95% confidence intervals (CIs). We estimated *p*-values using the equation from Bagchi et al. (2011) derived from Gelman & Hill (2007). The PCA was performed using the rda function in the vegan package.

Results

Soil water potential began to decline in the no watering treatment after approximately 45 days (Fig 1). This decline varied slightly among species with *S. macrophylla*, *D. lanceolata*, *D. oxleyanus* and *K. excelsa* having significantly faster decline in soil water potential than the rest of the species (Supplement). Although soil water potential remained relatively high in the infrequent watering treatment, pre-watering soil water potential was declining with days since the start of the treatment (slope = -0.0015 95% CI: -0.002 – -0.001) whereas post-watering soil water potential was constant (slope = 0.0001 95% CI: -0.001 – 0.001) implying every watering event was re-saturating the soil. No significant differences were found in soil temperature between treatments.

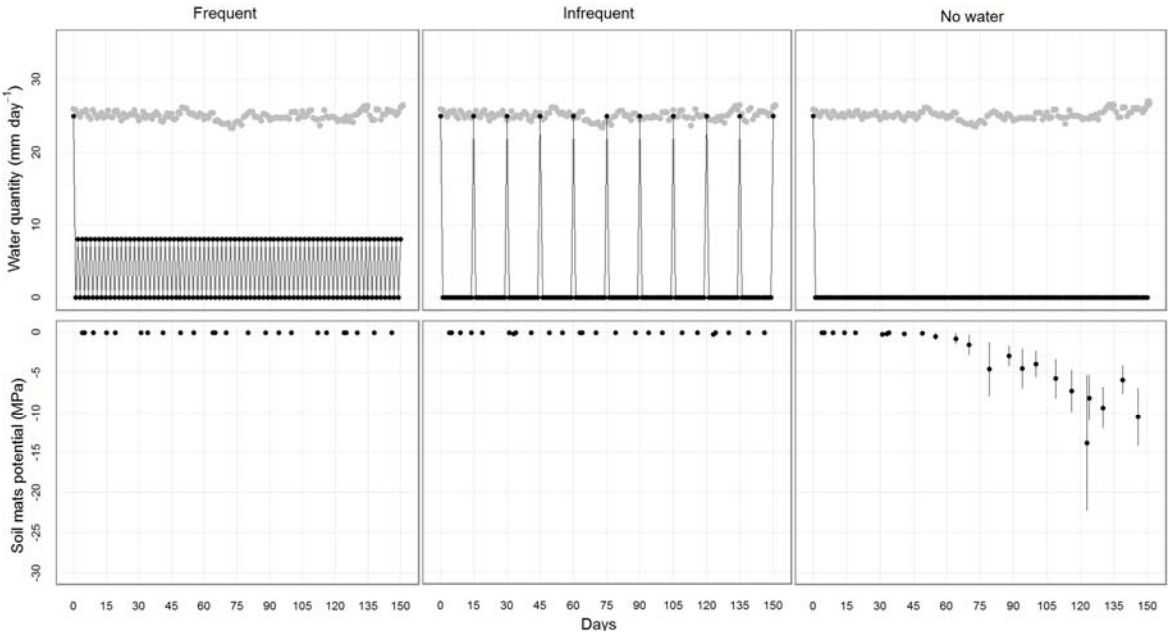

Fig 1 The watering pattern and soil water potential (mean ± 95% CI) of each treatment throughout the course of the experiment. The grey points represent mean daily soil temperature (degrees Celsius) for each treatment.

Seedling growth, daily stomatal conductance and leaf and stem water potentials all decreased with no watering, but non-structural carbohydrate concentrations increased in most species (Fig 2). Mortality was not significantly different between the control and infrequent watering treatment ($\beta_{frequent}$ = -0.1, 95% CI: -0.8 – 0.6 and $\beta_{infrequent}$ = -1.2, 95% CI: -2.5 – 0.4, respectively) but was significantly higher in the no watering treatment (β = 1.9, 95% CI: 1.2 – 2.6). Total mortality counts by species and treatments are in the supplement (Table S1).

Dieback varied among species with leaf loss beginning for *K. excelsa* first and *H. nervosa* last (Fig 3). The number of days until the start of dieback significantly varied in the no watering treatment from 90 days for *S. macrophylla* to 150 days for four species (*H. nervosa*, *P. tomentella*, *S. argentifolia* and *S. beccariana*). Dieback in the infrequent watering was significantly different from the frequent watering for only three species (*K. excelsa*, *S. beccariana* and *S. macrophylla*) after 150 days. However, all species had some dieback under infrequent watering except for *D. oxleyanus*, *H. nervosa* and *P. malaanonan*.

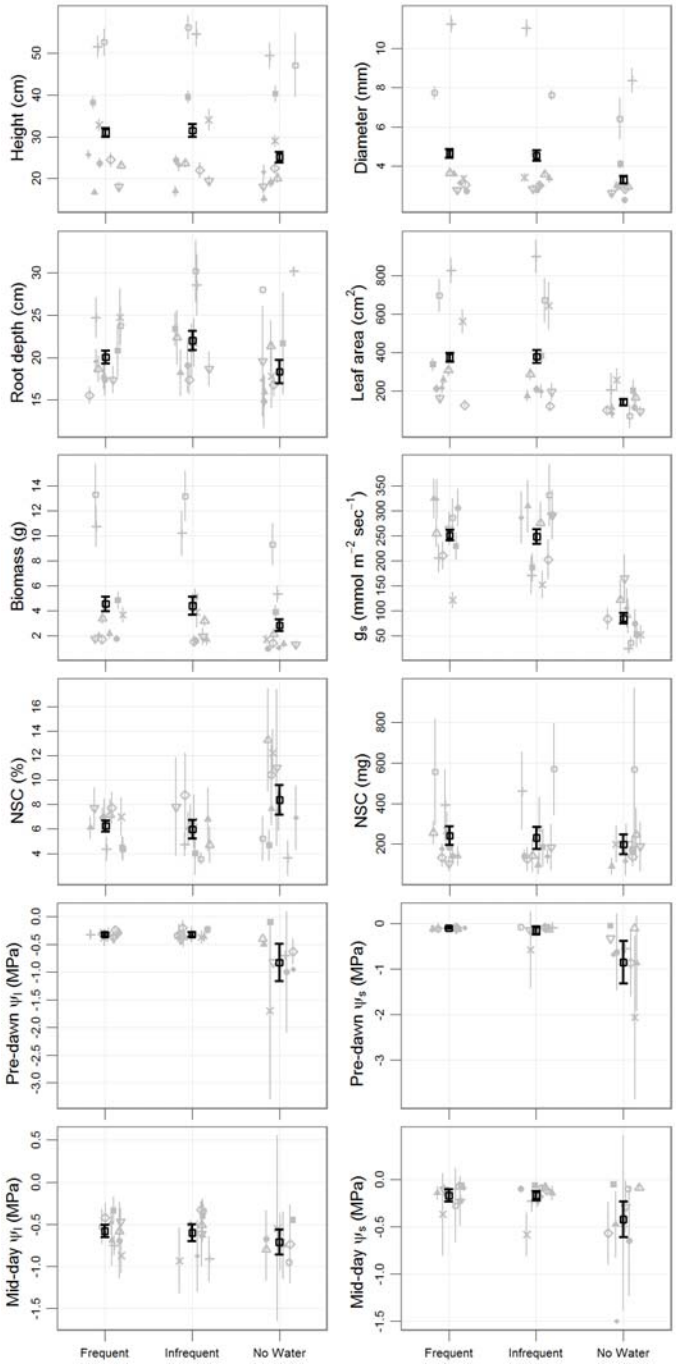

Fig 2 Growth and physiological traits influenced by watering treatment. Black squares represent mean (±95% CI) for all species and grey points represent the means (±95% CI) of each species (DO: cross, KE: x, SM: open circle, DL: closed square, PT: upward triangle, SP: closed diamond, SA: closed circle, PM: downward triangle, SB: closed triangle, HN: open diamond). Means were calculated from all data collected after 60 days since the beginning of the treatments. Species means are jittered for readability.

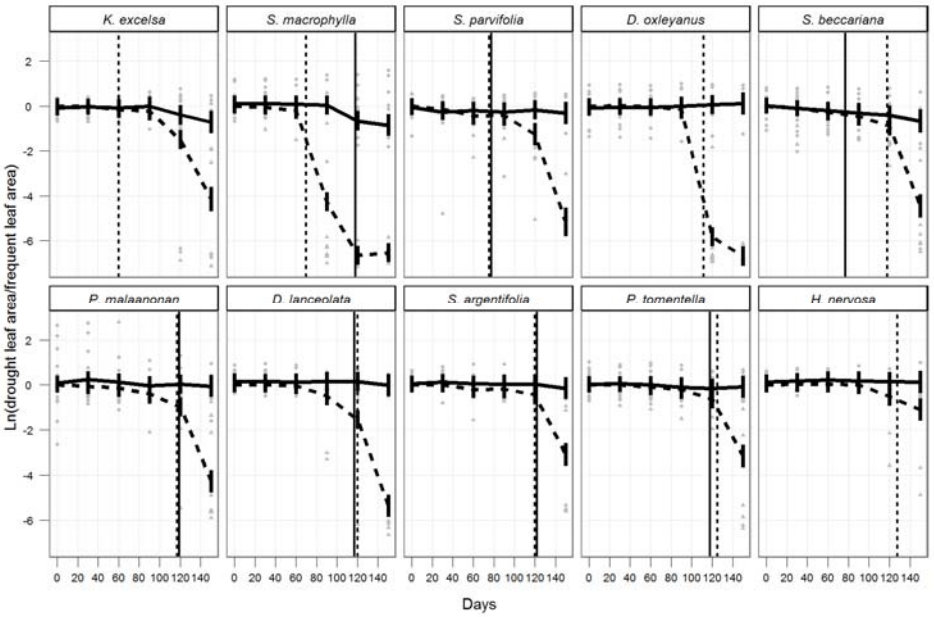

Fig 3 Leaf dieback (± 95% CI) as a function of days since the start of the experiment. Dieback was calculated as the natural log of the proportion of leaf area for an individual at each time point relative to the average leaf area for that species in the frequent watering treatment. The solid line represents the infrequent watering and the dashed line represents the no watering treatment, and the vertical lines represent the median days to leaf loss for each species. The grey points represent individual seedlings at each time point (infrequent: circles and no water: triangles). Species are ordered in terms of fewest to most days until median leaf loss in the no water treatment (top left to bottom right).

Leaf mass fraction (LMF) and shoot:root ratio (SRR) were significantly positively correlated with dieback (r^2 = 0.82, p = 0.003 and r^2 = 0.65, p = 0.04, respectively), and root mass fraction (RMF) was negatively correlated with dieback (r^2 = -0.58, p = 0.08) at the early stages of plant stress (i.e. 60 days of no water; Supplement). These allocation traits were also correlated with dieback after 150 days of infrequent watering (LMF: r^2 = 0.72, p = 0.02; SRR: r^2 = 0.64, p = 0.05 and RMF: r^2 = -0.64, p = 0.05; Fig 4). After more severe drought conditions (i.e. 150 days of no

water), physiological traits of non-structural carbohydrate concentration and sensitivity of xylem potential to soil water potential became correlated with dieback (r^2 = -0.69, p = 0.03 and r^2 = 0.58, p = 0.08, respectively; Fig 5). Surprisingly, greater root length was not correlated with dieback under mild drought stress and was positively correlated with dieback under severe drought (Table S2). The first two principal components (PC) explained 48.5% and 27.9% of the variability among species (Fig 6). Non-structural carbohydrate concentration (NSC) was strongly negatively correlated with PC1 while SGR, initial leaf area (LA_i), and $d\Psi_x$ /$d\Psi_s$ were strongly positively correlated with PC1. LMF was strongly negatively correlated with PC2 while RMF was strongly positively correlated with PC2.

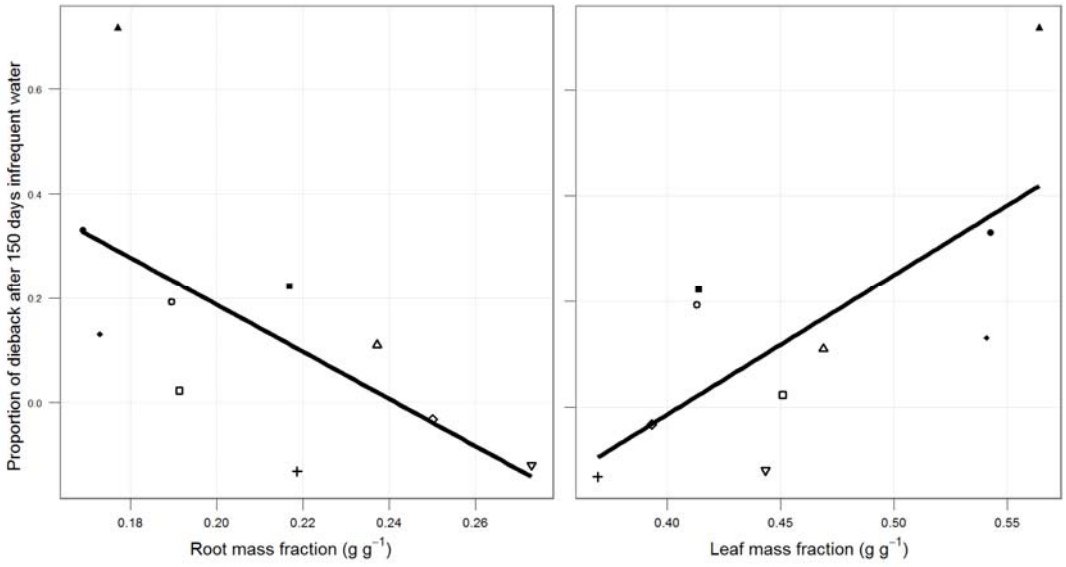

Fig 4 The relationship between proportion of dieback and root mass fraction and leaf mass fraction after 150 days of infrequent water. Lines represent direction of relationship and points represent mean values for each species (DO: cross, KE: x, SM: open circle, DL: closed square, PT: upward triangle, SP: closed diamond, SA: closed circle, PM: downward triangle, SB: closed triangle, HN: open diamond).

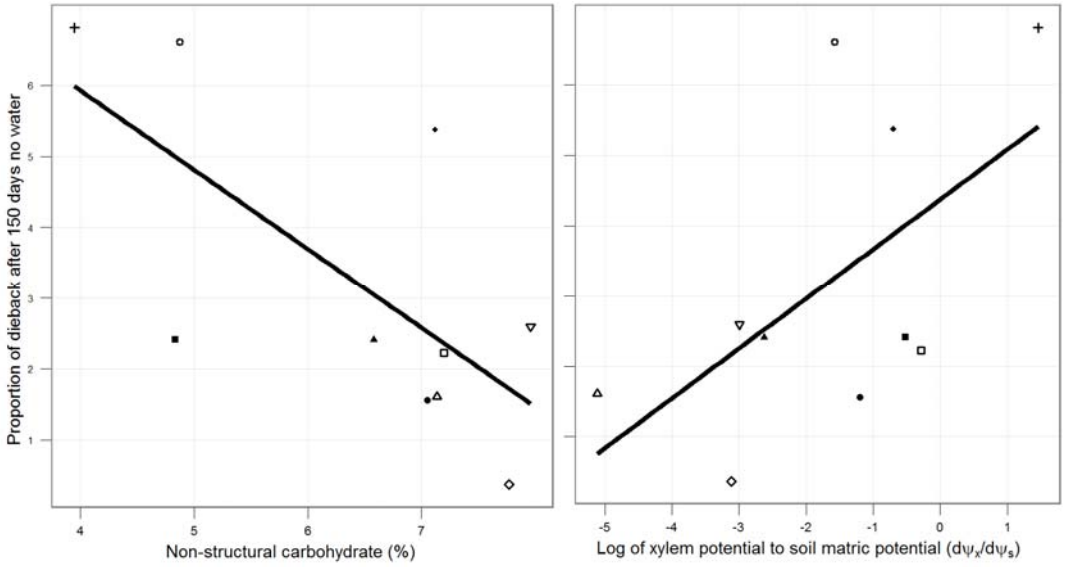

Fig 5 The relationship between proportion of dieback and non-structural carbohydrate concentration and sensitivity of mid-day xylem potential to soil water potential after 150 days of no water. The sensitivity of mid-day xylem potential was logged to make the data linear. Lines represent direction of relationship and points represent mean values for each species (DO: cross, KE: x, SM: open circle, DL: closed square, PT: upward triangle, SP: closed diamond, SA: closed circle, PM: downward triangle, SB: closed triangle, HN: open diamond).

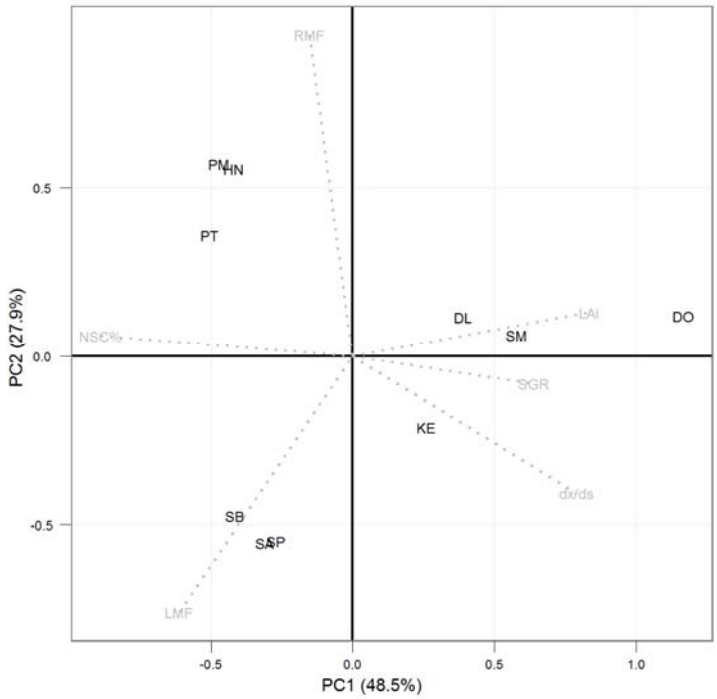

Fig 6 The results of the principal components analysis in two-dimensional space. The two principal component axes account for a total of 76.4% of the variability among species. The grey lines are trait vectors (RMF: root mass fraction, RLA: rooting depth:leaf area, NSC%: non-structural carbohydrate concentration, LMF: leaf mass fraction, LAR: leaf area ratio, SLA: specific-leaf area, SRR: shoot:root ratio, SGR: size corrected growth rate, dx/ds: change in xylem water potential with soil matric potential, and LAi: initial leaf area). The black text represents the species (the code is the first letter of the genus and specific epitaph).

Discussion

We examined the importance of morphological traits, biomass allocation and physiological response in mediating drought resistance of tropical forest tree species. Under mild drought stress, species with greater relative allocation to roots rather than shoots and leaves had less dieback. As drought stress became more severe due to prolonged deficits, physiological traits were more important for resistance. Species with the ability to maintain high xylem water potentials and those with greater NSC

concentrations had lower dieback. Under infrequent watering conditions and the first 90 days of no water, all species were able to maintain growth equal to that of frequent watering as well as high tissue water potentials which indicates these species were exhibiting behavior most similar to drought tolerators.

Species with lower leaf biomass and increased root biomass had less dieback under mild drought stress. Larger roots with reduced leaf area would allow maintenance of water conductance and water potentials because of access to soil water reserves and reduced transpiration (Hsiao 1973; Sperry et al. 2002; Markesteijn & Poorter 2009). These traits were important both after 60 days of water deficit as well as after 150 days of infrequent watering. However, these two aspects of drought affected species differently. For example, *S. parvifolia* had the greater dieback after 60 days of no water than under infrequent watering where it had less than half as much dieback (0.30 and 0.13, respectively). Alternatively, *S. beccariana* had more than three times higher dieback with infrequent watering than with no water (0.72 and 0.20, respectively). These results imply a differential tolerance of species to various characteristics of drought and rainfall patterns. Tolerance to anaerobic soils associated with flooding during extreme rainfalls may be an additional stress under infrequent rainfall that could explain these differences, although the underlying mechanisms were not identified in this study (Parolin 2001).

Species which sustained high xylem potentials had less dieback under severe drought. High xylem potentials would maintain leaf turgor pressure and prevent embolisms (Tyree et al. 1998). This characteristic was significantly negatively correlated with baseline SGR indicating slow growing species were better at maintaining high xylem potentials. Reduced gas exchange would both limit growth rates and water loss thereby increasing drought resistance. Loss of xylem conductivity

has been suggested as an important mechanism in desiccation induced mortality (Kursar et al. 2009).

Greater non-structural carbohydrate concentrations were significantly negatively correlated with drought dieback. Tolerance to other forms of stress such as herbivory and low light has been linked to plant NSC concentrations (Kobe 1997; Myers & Kitajima 2007; Poorter & Kitajima 2007). Reduced dieback in species with greater NSC concentrations was likely indirectly due to a storage/growth trade-off (Poorter & Kitajima 2007). The PCA showed a partitioning of species on the PC1 axis with fast growing species separated from species with high NSC (Fig 6). Slower growth and higher NSC concentrations indicated a more conservative strategy which improved drought tolerance. Additionally, the most sensitive species to water deficit accumulated far less NSC under drought than the most tolerant (*S. macrophylla* increased 0.3% while *H. nervosa* increased 2.3%). Therefore, both baseline NSC concentrations as well as the ability to allocate NSC to storage instead of growth during drought were important for reducing dieback. However, this study could not elucidate the functional role of NSC for drought tolerance, and further studies are necessary for understanding the mechanisms in which NSC concentration provide drought tolerance.

Summarizing all the results together allowed us to categorize the species into four responses: 1) greater sensitivity to deficits (*D. oxleyanus*, *K. excelsa*, *P. malaanonan* and *S. parvifolia*), 2) greater sensitivity to cycles of short-term drought followed by flooding (*S. argentifolia* and *S. beccariana*), 3) equal sensitivity to both (*S. macrophylla* and *D. lanceolata*) and 4) equal tolerance to both (*H. nervosa* and *P. tomentella*). These general categories may explain landscape level species distributions. For example, Born (2011) found *S. argentifolia* to be highly sensitive to

flooding but resistant to dry periods. Furthermore, Paoli et al. (2006) found that *S. macrophylla* and *S. parvifolia*, more susceptible species, were positively associated with rich alluvial soils and absent from more coarse textured granite soils whereas a *Hopea* sp. was associated with granite (*H. nervosa* was one of the most resistant). *D. oxleyanus* and *S. macrophylla* (larger seeded species in general) have been shown to associate with high clay content soils which retain water longer and are both considered riparian species which experience frequently flooded soils (Wood & Meijer 1964; Lemmens et al. 1995; Soepadmo & Wong 1995; Potts 2003; Katabuchi et al. 2012).

Conclusion

These results are consistent with niche partitioning of Bornean species across a drought gradient from highly susceptible to highly resistant with multiple traits playing a functional role in explaining this partitioning. Drought as a niche axis likely plays a role in landscape level species distributions. We would predict species to distribute across soil types with slow growing and high NSC concentration species on well drained sandy soils, fast growing and low NSC concentration species on poorly drained clay soils and species with small relative leaf biomass and large relative root biomass to dominate moderately clayey soils with mild levels of water stress.

Acknowledgements

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Supplement

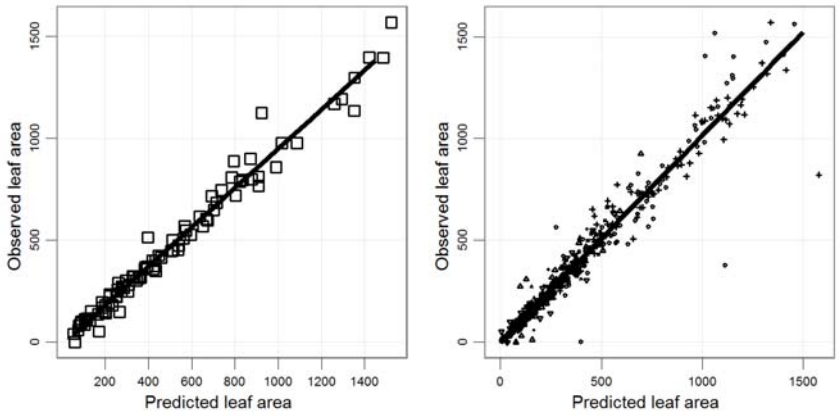

Fig S1 Model fits of leaf area for *K. excelsa* (left panel) and all other species (right panel). Because *K. excelsa* had a composite leaf with many leaflets, the equation *la = length*width* was used to estimate individual leaflet area and then scaled up to total leaf area by multiplying mean leaflet area by the number of leaflets. Total plant leaf area was the sum of all leafs ($R^2 = 0.98$, n = 89). The leaf area of all other species was calculated with the equation *la = species + length + width + length*width*. Total plant leaf area was the sum of all leaves for an individual plant ($R^2 = 0.95$, n = 783). Outliers were due to poor estimates of dieback.

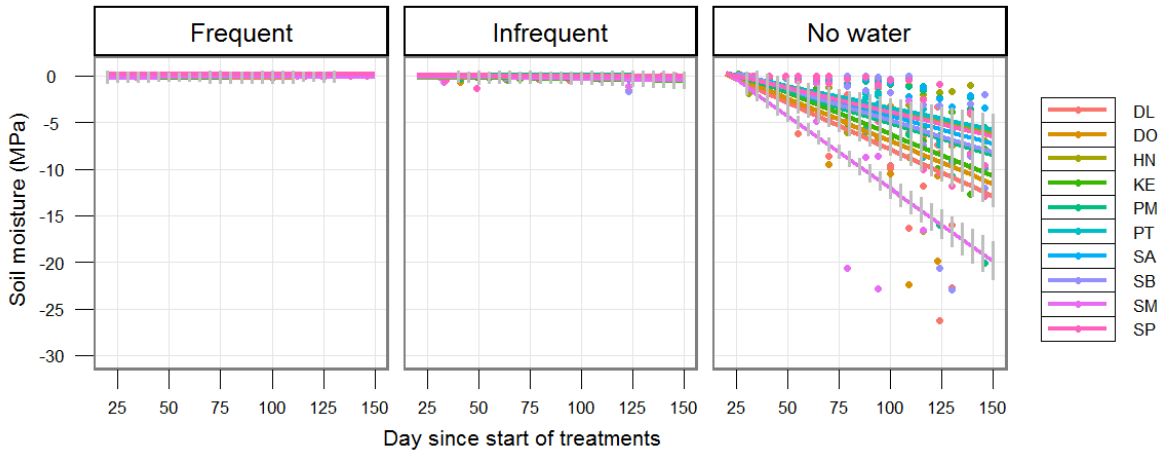

Fig S2 Soil water potential as a function of days since the start of the treatment for each species. Lines represent the model fit, and points represent observations. SM, DL, DO and KE all declined significantly faster than the rest of the species.

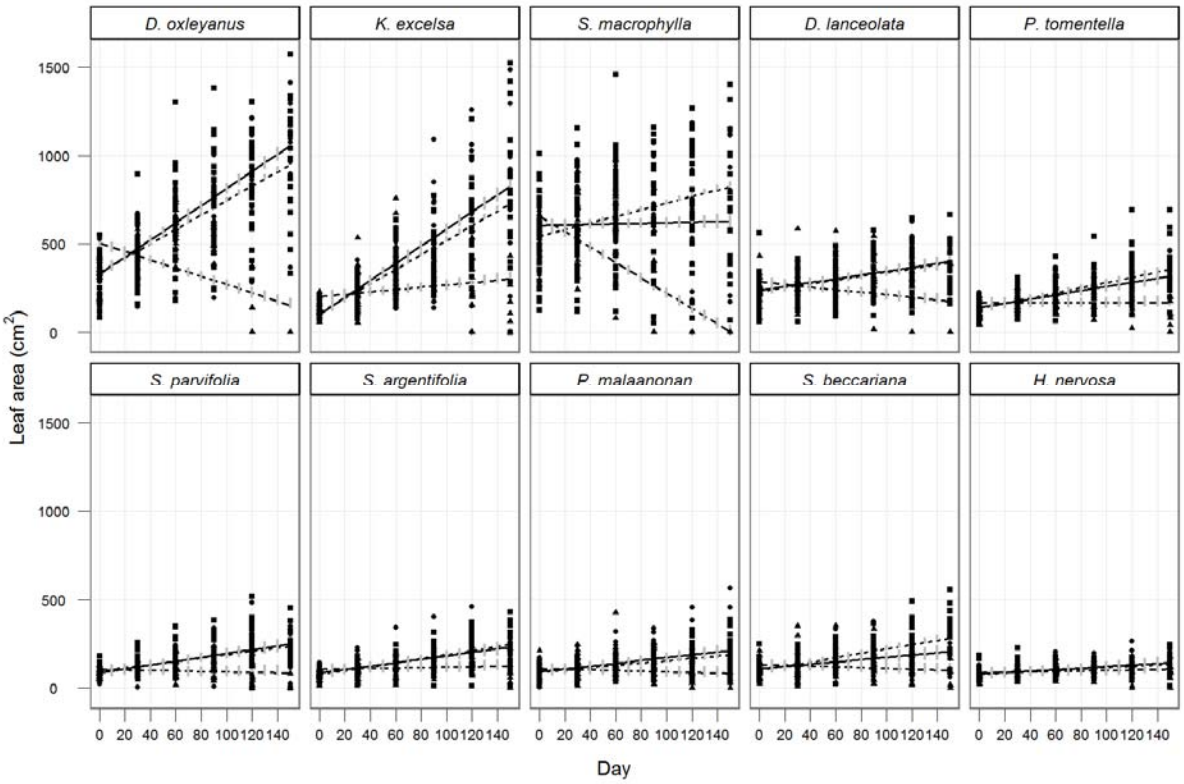

Fig S2 Leaf area growth as a function of time for each species in each treatment. These curves were used to calculate size-specified growth rates (SGR) for each species. SGR was calculated at 125 cm^2, the minimum leaf area observed for all species. The lines represent model predictions with 95% CI (frequent water: small dash, infrequent: solid line and no water: dashed line), and the points represent measurements from individual seedlings (frequent: squares, infrequent: circles and no water: triangles). The species are in order from the highest RGR (top left) to the lowest RGR (bottom right) in the frequent watering treatment.

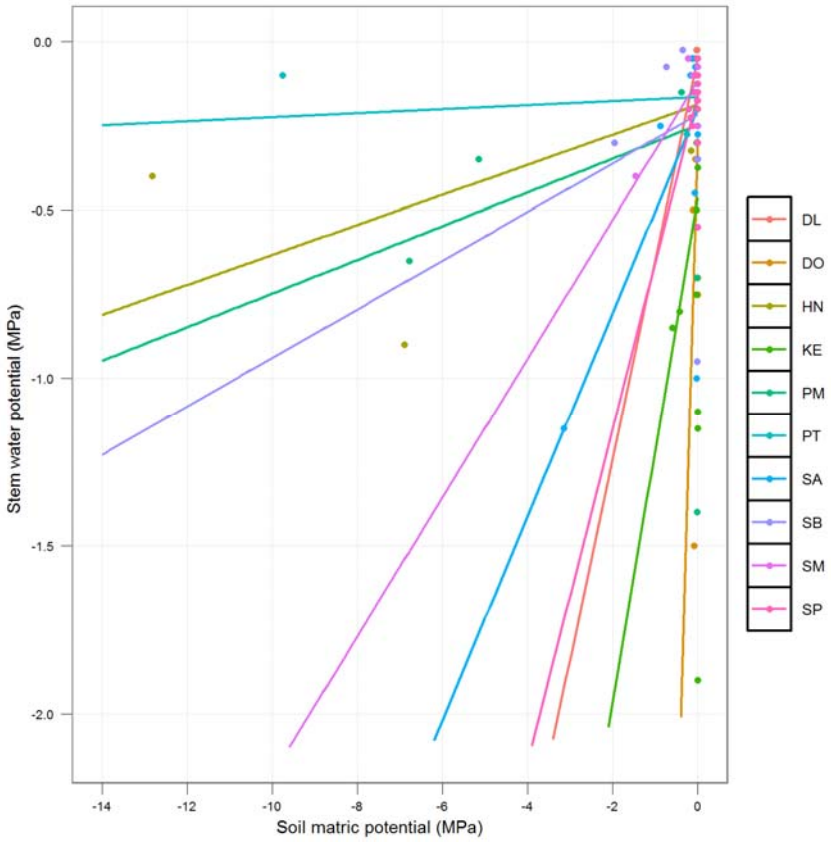

Fig S3 Mid-day stem water potential as a function of soil water potential. The lines represent the model fit for each species and the points represent observations. The slope of each line was used as a proxy for the sensitivity of xylem potential to low soil water potential ($d\Psi_x / d\Psi_s$).

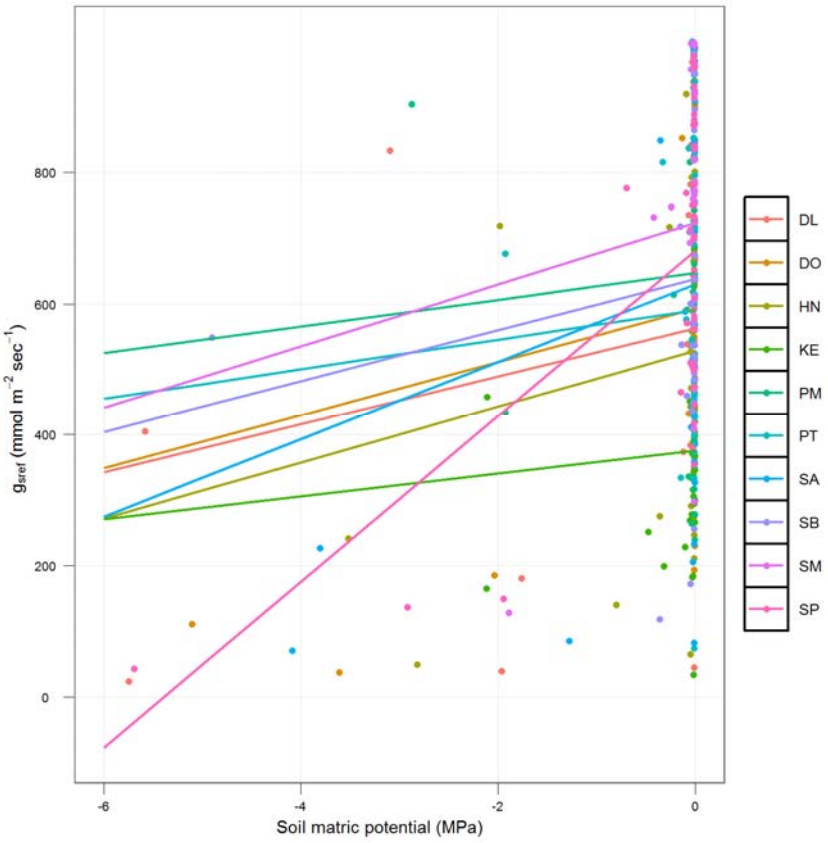

Fig S4 Mean daily stomatal conductance as a function of soil water potential. The lines represent the model fit for each species and the points represent observations. The slope of each line was used as a proxy for the sensitivity of stomatal conductance to decreasing soil water potential ($dg_s/d\Psi_s$).

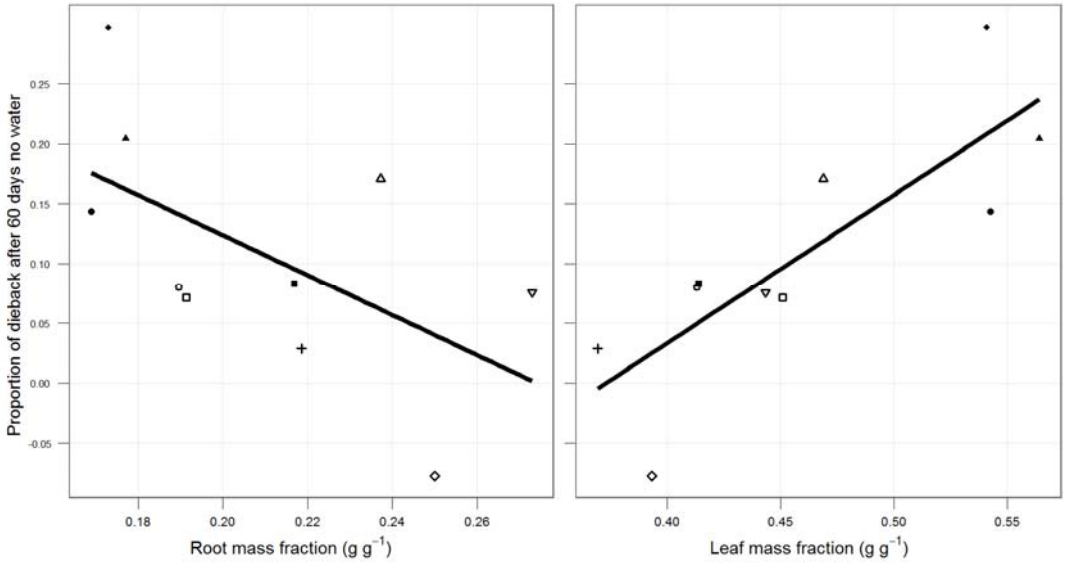

Fig S5 The relationship between proportion of dieback and root mass fraction and leaf mass fraction after 60 days of no water. Lines represent direction of relationship and points represent mean values for each species (DO: cross, KE: x, SM: open circle, DL: closed square, PT: upward triangle, SP: closed diamond, SA: closed circle, PM: downward triangle, SB: closed triangle, HN: open diamond).

Table S1 Total mortality counts by species and treatment.

Species	Frequent	Infrequent	No water
D. lanceolata	1	0	9
D. oxleyanus	0	0	13
H. nervosa	0	0	2
K. excelsa	1	1	3
P. malaanonan	0	0	6
P. tomentella	0	0	3
S. argentifolia	1	0	5
S. beccariana	3	1	6
S. macrophylla	1	1	12
S. parvifolia	3	0	6

Table S2 Pearson correlation coefficients between mean growth and allocation traits from frequent watering treatment and mean dieback at four time points. Bold values are p-values < 0.10 and bold italic values are p-values < 0.05.

	No Water				**Infrequent water**			
Days since treatment began	60 days	90 days	120 days	150 days	60 days	90 days	120 days	150 days
RLA (mm cm^{-2})	0.00	-0.38	-0.52	**-0.56**	-0.11	0.24	0.04	0.04
SLA (cm^2 g^{-1})	-0.01	-0.34	-0.35	-0.20	-0.20	-0.26	-0.41	-0.19
SRR (g g^{-1})	***0.65***	0.36	0.16	0.29	0.45	0.23	0.33	***0.64***
RMF (g g^{-1})	**-0.58**	-0.37	-0.21	-0.29	-0.48	-0.20	-0.34	***-0.64***
LMF (g g^{-1})	***0.82***	-0.04	-0.37	-0.22	**0.63**	***0.68***	**0.57**	***0.72***
LAR (cm^2 g^{-1})	0.31	-0.30	-0.45	-0.26	0.06	0.03	-0.15	0.12
Leaf area (cm^2)	-0.32	0.69	***0.88***	***0.73***	-0.32	-0.55	-0.32	-0.34
Root depth (mm)	-0.08	0.45	***0.67***	***0.72***	-0.15	-0.41	-0.10	-0.20
SGR (cm^2 cm^{-2} day^{-1})	-0.21	-0.18	0.06	0.29	-0.28	-0.43	-0.51	-0.42

Table S3 Pearson correlation coefficients between mean physiological traits and mean dieback at four time points. NSC values were calculated from frequent watering treatment, and sensitivity of xylem pressure ($d\Psi_x / d\Psi_s$) and stomatal conductance ($dg_s/d\Psi_s$) to decreasing soil water potential were calculated from all measured data. Bold values are *p-values* < 0.10 and bold italic values are *p-values* < 0.05.

	No Water				**Infrequent water**			
Days since treatment began	60 days	90 days	120 days	150 days	60 days	90 days	120 days	150 days
NSC concentration (%)	0.13	-0.45	***-0.65***	***-0.69***	0.11	-0.16	0.01	0.03
NSC pool (mg)	-0.10	***0.85***	***0.96***	***0.69***	0.14	0.33	-0.15	-0.01
Log $d\Psi_x / d\Psi_s$	0.08	0.15	0.29	**0.58**	-0.16	-0.46	-0.52	-0.16
Log $dg_s/d\Psi_s$	0.43	0.18	0.14	0.39	0.28	0.06	0.06	0.25

Table S4 Pearson correlation coefficients between traits. Bold values are *p-values* < 0.05.

	RLA	SLA	SRR	RMF	LMF	LAR	Leaf	Root	NSC (%)	NSC (mg)	$d\Psi_x/d\Psi_s$	$dg_s/d\Psi_s$
RLA ($mm\ cm^{-2}$)	NA											
SLA ($cm^2\ g^{-1}$)	-0.23	NA										
SRR ($g\ g^{-1}$)	-0.22	0.15	NA									
RMF ($g\ g^{-1}$)	0.39	-0.20	**-0.98**	NA								
LMF ($g\ g^{-1}$)	0.33	0.12	**0.71**	-0.61	NA							
LAR ($cm^2\ g^{-1}$)	-0.06	**0.92**	0.42	-0.42	0.50	NA						
Leaf Area (cm^2)	**-0.76**	-0.11	0.01	-0.12	-0.61	-0.33	NA					
Root depth (mm)	**-0.72**	-0.17	-0.20	0.11	-0.58	-0.38	**0.79**	NA				
NSC (%)	0.74	0.30	-0.12	0.22	0.45	0.45	**-0.81**	**-0.67**	NA			
NSC (mg)	-0.65	-0.33	0.17	-0.26	-0.37	-0.44	**0.90**	**0.72**	**-0.70**	NA		
$d\Psi_x/d\Psi_s$	-0.47	0.04	-0.07	0.04	-0.43	-0.16	0.48	0.57	-0.63	0.25	NA	
$dg_s/d\Psi_s$	0.04	-0.16	0.61	-0.53	0.44	0.04	-0.18	-0.06	0.02	-0.05	0.04	NA
SGR ($cm^2\ cm^{-2}\ day^{-1}$)	**-0.64**	**0.72**	-0.03	-0.07	-0.34	0.48	0.42	0.42	-0.33	0.09	**0.65**	-0.25

Chapter 4

Experimental manipulation of non-structural carbohydrate concentrations demonstrates a general effect on drought resistance both within and among species of tropical trees

Michael J. O'Brien, Sebastian Leuzinger, Christopher D. Philipson, Philip Ulok, John Tay & Andy Hector

Abstract

Tropical forests are under threat from global change, with increased drought through climate alteration a major concern. Inference on the effect of climate induced drought on the distribution and persistence of species depends upon intra- and inter-specific diversity in traits providing a potential insurance effect. Non-structural carbohydrate (NSC) storage is one important trait for plant survival against drought and other abiotic and biotic stresses. Here we use a novel experimental approach to manipulate NSC concentrations while controlling for differences in plant size, a confounding factor in many previous studies. We show that within species survival was on average 9.2 days longer for NSC enriched seedlings than for NSC depleted seedlings, and within species variation in NSC concentrations was correlated with variability in time to death. Across all species, increasing NSC concentrations reduced the risk of mortality while delaying the time to death. However, initial levels of NSC concentration after their experimental manipulation did not affect subsequent growth. The importance of NSC concentration in seedling resistance to drought is highlighted by the unanimous increase in time to death across all species. Intra-specific plasticity in key traits allows the expansion of climatic envelopes with climate change, and we suggest that variation in NSC concentrations may provide an insurance effect against climate change induced drought. This effect may allow evolutionary adaptation to climate change and persistence of species in altered climate envelopes. Nevertheless, since species vary in inherent NSC storage, the presence of species with higher levels of NSC in communities could provide an insurance effect to buffer against the increased intensity and frequency of drought projected for tropical forests

Keywords

Plant-climate interactions; Stress tolerance; Intra-specific variability; Carbon storage; Climate change

Introduction

Precipitation patterns are changing throughout the globe altering current climate zones, causing forest die-back and affecting ecosystem function (Potts 2003; Williams et al. 2007; Phillips et al. 2010; Lewis et al. 2011; Anderegg et al. 2012; Choat et al. 2012). Within species variation, whether the consequence of genotypic variability or phenotypic differences due to local environmental filters, may act as an insurance effect against species extinction under a changing climate (Loreti & Oesterheld 1996; Clark et al. 2011; Clark et al. 2012; Rapp et al. 2012). Identifying traits that promote plant resistance to drought, both within and among species, is therefore important for predicting the effect of climate change on species persistence in altered and novel climates (Williams et al. 2007; Buckley & Kingsolver 2012).

Carbon storage is intrinsically tied to the stress tolerance of plants against drought, herbivory and shade (Chapin et al. 1990; Kobe 1997; Myers & Kiajima 2007; Poorter & Kitajima 2007; Adams et al. 2009; McDowell et al. 2011; Sala et al. 2012). Non-structural carbohydrate (NSC) concentrations play a functional role in the maintenance of plant metabolism and resistance to and repair of tissues during stress (Bucci et al. 2003; Myers & Kiajima 2007; Sala et al 2012). For example, there is strong evidence for the importance of NSC in drought tolerance (Anderegg et al. 2012). Under moderate drought, NSC is important for maintaining cell turgor (Hsiao 1973), and in more severe drought conditions, NSC pools may be important for refilling embolisms and maintaining vascular integrity (Bucci et al. 2003; Zwieniecki

& Holbrook 2009; Brodersen et al. 2010; Sala et al 2012). This process implies that NSC concentrations are actively sustained in order to buffer plants during stochastic stress events (Sala et al. 2012; Wiley & Helliker 2012). However, most research has focused on among species differences in stress tolerance (Kobe 1997; Myers & Kitajima 2007), and the effect of stress on NSC depletion and/or accumulations (Würth et al. 2005; McDowell et al. 2008; Adams et al. 2009; Sala & Hoch 2009; Woodruff & Meinzer 2011; Anderegg et al. 2012). Differences in intra-specific carbon storage and its role in stress tolerance has received far less attention due to the difficulty in sampling NSC concentrations for many individuals and in controlling NSC concentrations without altering within species traits.

We use a novel experimental design to manipulate within species NSC concentrations while controlling differences in plant size. We track seedling mortality under drought of NSC rich and NSC depleted seedlings in order to test whether NSC concentration effects survival. We show that seedlings with higher NSC concentrations live longer than those with lower NSC concentrations, and within species variation in NSC concentration is correlated with within species death rates. This result is consistent at the both the intra- and inter-specific level.

Methods

Study site

We conducted this experiment at the Sabah Biodiversity Experiment (N05º05'20'' E117º38'32''; 102 MASL). This site is located ≈22 km north of Danum Valley Research Center in Borneo Malaysia (Hector *et al*. 2011). Mean annual rainfall (SE) recorded in Danum Valley from 1986-2010 was 2848.5 (94.0) mm. Daily temperature during the course of the experiment averaged 25.5 C (0.02) with

average maximum and minimum temperatures of 30.0 C and 23.0 C, respectively. The average (SE) daily humidity was 92.6% (0.1) with average maximum and minimum humidity of 98.4% and 74.2%, respectively. Measurements of climatic variables were collected hourly using digital sensors (SKH 2000 probe, Skye instruments LTD, Llandrindod Wells, Powys, UK).

Seedlings

Seeds from 10 species of shade-tolerant trees were collected during a landscape scale masting event in August 2010. We collected seeds directly from three different mature trees for each species with the exception of *Durio oxleyanus* and *Hopea nervosa* which were each obtained from only one tree. Seeds were not planted on the same day because seed maturation varied among species, but all seeds were planted between the 10th and 29th of August 2010. We placed each seed under wet burlap sacks to germinate. After germination, each seed was planted into pots (20 x 36 cm) within a nursery. Pots were filled with homogenized forest soil obtained from the Innoprise-FACE Foundation Rainforest Rehabilitation Project (http://www.face-thefuture.com). The soil was classified as clay comprised of 50% clay, 30% silt and 20% sand. We planted 46-47 seeds from each mature tree for a total of 140 seeds per species. Seedlings were raised in a nursery until every individual had dropped their cotyledons. We replanted individuals that died during the first two months from nursery grown seedlings, of the same mature tree.

Experimental manipulation of non-structural carbohydrate concentrations

On 14 December 2010 seedlings were placed in one of two nurseries: high light or low light (70 individuals x 10 species = 700 individuals in each nursery). The high light nursery was covered with one layer of 70% shadecloth and averaged (SE)

26.4% (1.3) of direct sunlight. The low light nursery was covered with three layers of 70% shadecloth and averaged (SE) 0.4% (0.04) of direct sunlight. Light was measured by simultaneous nursery and open sky photosynthetically active radiation sensors (SKP 210 quantum sensor; Skye instruments LTD, Llandrindod Wells, Powys, UK) for a 24-hour period at each of three locations in each nursery (i.e. 72 hours of measurement per nursery). Species were mixed within each nursery so that variations in the light and climatic environment were equally distributed across all species. After 99 days all seedlings were moved to the alternate light environment. After a second 99 day period, all seedlings were measured for height and diameter and 120 seedlings of each species were randomly assigned to one of 15 shadehouses. The shadehouses consisted of two layers of 70% shadecloth. Below the shadecloth clear plastic polyethylene sheeting was used to exclude rainfall. This design provided the seedlings with an average (SE) of 9.5% (1.1) light and a red:far-red ratio (SE) of 1.1 (0.03) measured nursery measurements. Following 198 days, seedlings either received a low–high light treatment (High-NSC) or a high–low light treatment (Low-NSC) in which NSC concentrations were significantly higher in the High-NSC treatment ($\beta_{High\text{-}NSC}$ = 1.84%, 95% CI: 1.17 – 2.15%; Fig. S1). However, leaf area ($\beta_{High\text{-}NSC}$ = -47.3 cm^2, 95% CI: -97.8 – 3.3 cm^2), diameter ($\beta_{High\text{-}NSC}$ = -0.01 mm, 95% CI: -0.13 – 0.11 mm) and height ($\beta_{High\text{-}NSC}$ = -2.3 cm, 95% CI: -3.4 – -1.2 cm) were biologically equal between the Low-NSC and High-NSC treatment (Fig. S2).

Drought treatment

Sixty seedlings of each species were assigned to a drought treatment with no water (30 Low-NSC and 30 High-NSC), and sixty seedlings were given an average rainfall treatment with 240 mm of water per month on two day intervals (30 Low-NSC and 30 High-NSC). Five additional seedlings of each species from each NSC

treatment were harvested and used to assess pre-drought biomass, leaf area and NSC concentrations. We monitored the seedlings every two days for changes in leaf morphology and mortality.

Seedling growth, biomass and leaf area

We measured the height and diameter prior to the start of the drought treatment and approximately every month thereafter. We destructively harvested seedlings prior to the start of the drought treatment and 35, 83, 102 and 123 days after the treatment began. We harvested one seedling of each species in each treatment at pre-dawn (03:00-06:00) and mid-day (12:00-14:00). We measured a number of traits of each seedling at each destructive harvest. In order to examine leaf senescence, we measured chlorophyll content on one leaf from the top, middle and bottom part of the crown of each seedling using a spadmeter (model Spad-502, Konica Minolta Sensing, Inc., Osaka, Japan). To assess the water status of each seedling, we measured leaf water potential on a single leaf (or a cluster of leaves for small seedlings) and stem water potential using a Scholander pressure chamber (model 670, PMS Instrument Co., Corvallis, Oregon, USA). We took photographs of all the leaves and analyzed their leaf area using ImageJ software (Rasband 2012). To assess rooting depth, we removed all soil and measured longest root length. In order to assess biomass, seedlings were dried at 64 C until a constant weight, and we weighed leaves, stem and roots separately.

Environmental conditions

In order to measure temperature differences between treatments, we placed Thermocron Ibuttons (model DS1921G-F5#, Maxim Integrated Products, Inc., Sunnyvale, CA, USA) in each NSC treatment in drought and watered treatments.

There was no measurable difference between the Low-NSC treatment ($\beta_{Low\text{-}NSC}$ = 25.5, 95% CI: 24.5 – 26.3) and the High-NSC treatment ($\beta_{High\text{-}NSC}$ = 25.5, 95% CI: 24.6 – 26.4). Volumetric soil moisture content at the top and bottom of the pot was measured on 1-2 seedlings of each species in each treatment every week with an ML2x Theta Probe and HH2 moisture meter (Delta-T Devices, Burwell, Cambridge, UK), to ensure equal soil drying between NSC treatments in the drought treatment. The relationship between soil matric potential (Ψ) and volumetric soil moisture content was determined using the filter paper method (Deka et al. 1995). No differences were found in soil drying through time between the High-NSC treatment (β_{slope} = -0.11, 95% CI: -0.13 – -0.10) and the Low-NSC treatment (β_{slope} = -0.12, 95% CI: -0.14 – -0.10).

Non-structural carbohydrate analysis

We used 5 seedlings of each species from each light manipulation to quantify NSC concentrations and pools (concentration x organ biomass) in the leaf, stem and roots in order to assess whether the light manipulations significantly altered stored NSC between individuals. We ground the organs of each seedling with a ball mill and used 15-16 mg of sample for NSC analysis. We extracted soluble sugars with a shaking bath of 80% ethanol at 27 C for one night followed by two additional 2-hour baths (Marquis et al. 1997; Myers & Kitajima 2007). We digested the remaining starch with amyloglucosidase (A-7420, Sigma-Aldrich, Co., St. Louis, MO, USA). The concentrations of simple sugars and starch were measured at 487 nm by spectrophotometry after a phenol-sulphuric acid reaction (Dubois et al. 1956; Ashwell 1966; Boege 2005).

Statistical analysis

We used a Cox proportional hazard model to assess differences in survival between Low- and High-NSC treatment seedlings within a species. This method was appropriate because monitoring every two days limited the number of ties and some seedlings were harvested before death, which caused left censoring. We modeled the probability of survival through time as a function of species identity, NSC level (high and low concentration) and their interaction. We stratified the data between watered and drought treatments to account for different disjoint baseline hazard functions between these two treatments (Therneau & Grambsch 2000).

We examined the effect of NSC concentrations on among species mortality with three metrics. First, we extracted the risk of mortality from the survival analysis for each species in each NSC treatment modeled as a function of mean NSC concentration for each species in each treatment. Second, we performed the same analysis as above but used days to death as the response variable instead of extracted mortality risk. Third, we modeled variability in time to death within a species as a function of variability of NSC concentrations within a species.

We analyzed height growth as a function of days since treatment began (day), species identity, NSC treatment, watering treatment and the interactions. Individual seedling was used as a random effect to account for individual variability in time and space. Due to correlation between repeated measures, we used an auto-regressive correlation matrix. We used Bayesian Information Criterion for model selection. Support for an effect of NSC concentration on growth would require that an interaction between days since the start of the treatment and NSC treatment would be retained in the selected model. All analysis was done the R statistical software

package (2.15.1; R Core Team 2012) with the nlme and survival packages (Pinheiro & Bates 2000; Therneau & Grambsch 2000).

Results

Seedlings from the High-NSC treatment survived 9.9 (95% CI 3.8 – 16.0) days longer on average than seedlings from the Low-NSC treatment (Fig 1). High NSC concentrations had the greatest effect on *D. lanceolata* survival which lived on average 21 days longer than conspecifics from the Low-NSC treatment while NSC enriched *P. malaanonan* seedlings only survived an average of 3 days longer than their NSC depleted counterparts. Cox proportional hazard analysis showed seedlings from the Low-NSC treatment had a 1.7 (95% CI: 1.4 – 2.1) times greater risk of dying than seedlings from the High-NSC treatment (Fig 2).

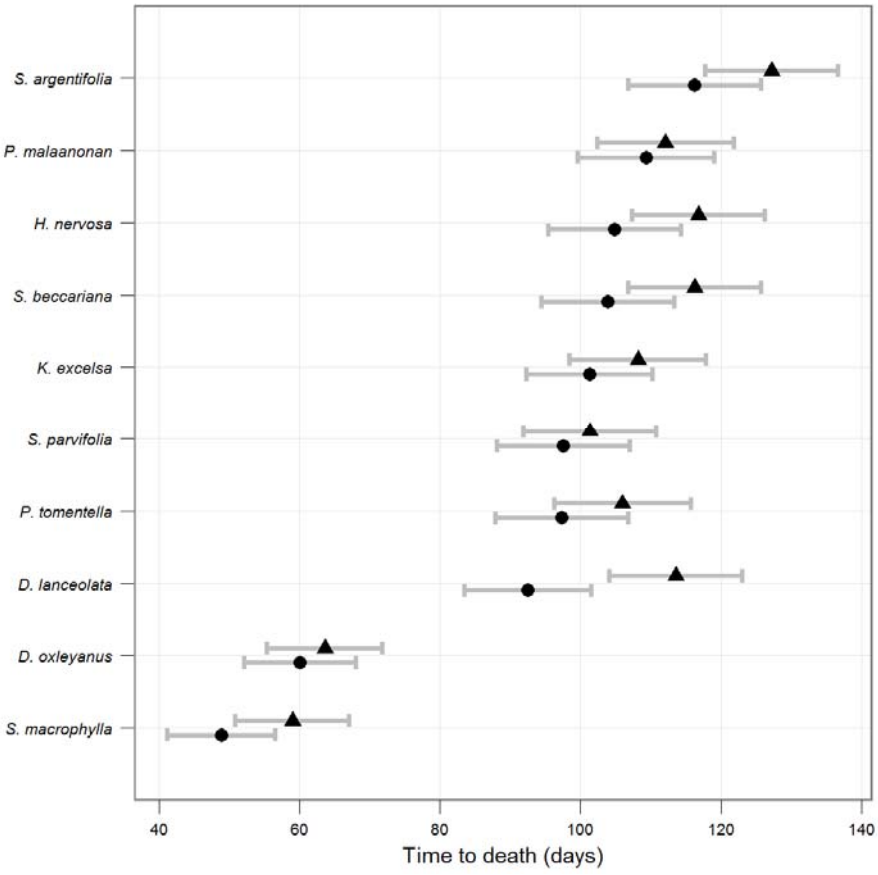

Fig 1 The mean days to death (95% CIs) for each species from each NSC treatment (Low-NSC: circles and High-NSC: triangles).

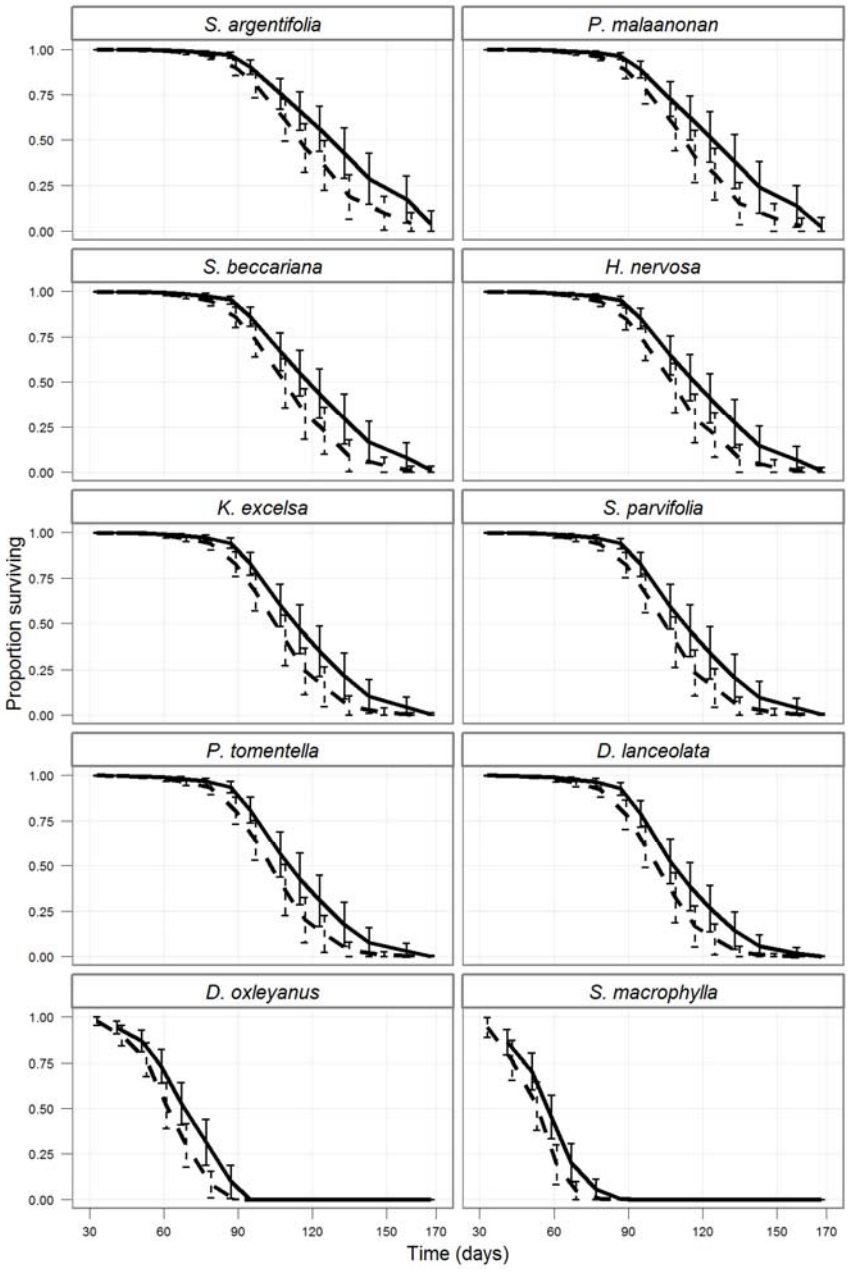

Fig 2 Proportion surviving as a function of time for low and high non-structural carbohydrate concentrations (NSC). Lines represent fits from a Cox proportional-hazard model with 95% CIs. Dashed lines represent Low-NSC, and solid lines represent High-NSC. Species are ordered in terms of rate of decline from slowest (top-left) to fastest (bottom-right).

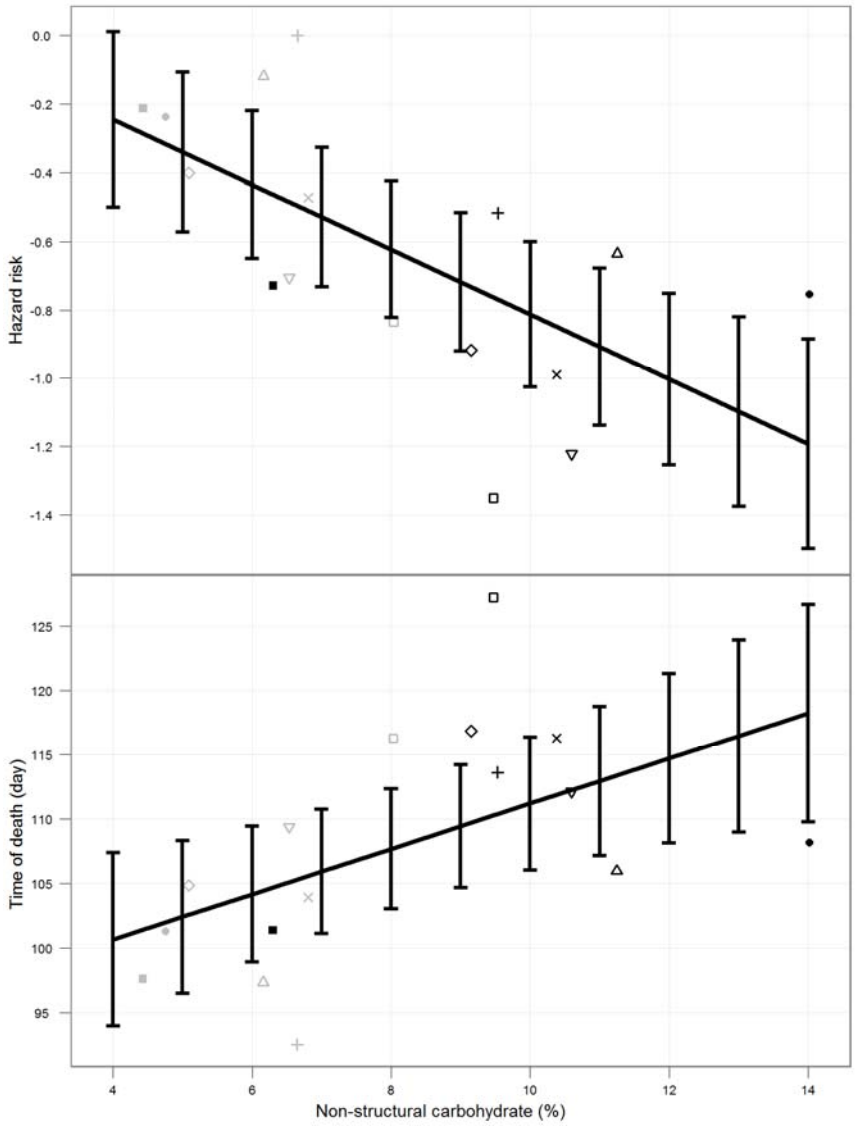

Fig 3 Hazard risk (extracted from the Cox proportional hazard model) and time to death modeled as a function of mean non-structural carbohydrate concentration of species from each NSC treatment. The line represents model fit with 95% CIs. Grey points represent mean values for each species from the Low-NSC treatment, and black points represent mean values for each species from the High-NSC treatment. (DL: cross, KE: closed circle, PT: upward triangle, SP: closed diamond, SA: open square, PM: downward triangle, SB: x, HN: open diamond).

Initial NSC of species prior to the drought was strongly correlated to hazard risk (Spearman correlation coefficient = -0.70, $p < 0.001$) and mean days to death (Spearman correlation coefficient = 0.68, $p < 0.01$), which indicates that among species NSC differences also correlate to survival. Because *S. macrophylla* and *D. oxleyanus* had substantially higher risk than all other species, we removed their data points to analyze risk and time to death as functions of NSC concentration with linear regression. Risk declined with NSC concentration (β_{slope} = -0.1, 95% CI: -0.14 – -0.05), and time to death increased with NSC concentration (β_{slope} = 1.8, 95% CI: 0.3 – 3.2; Fig 3). Variability in time to death directly increased with variability in NSC concentration (β_{slope} = 1.6, 95% CI: -0.3 – 3.5; Fig 4).

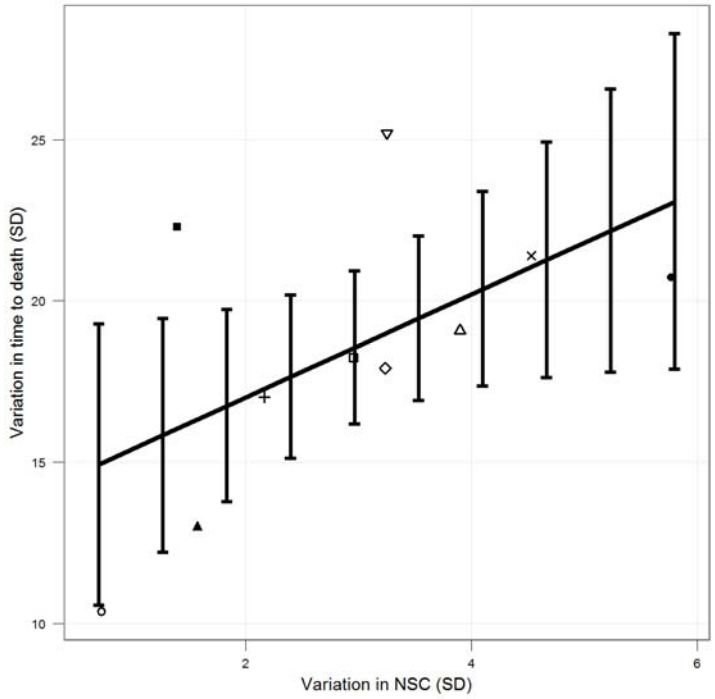

Fig 4 Variability (SD) in days to death as a function of variability in NSC concentration for each species. Solid line represents model fit (95% CIs), and points represent observed values for each

species. (DL: cross, KE: closed circle, SM: open circle, DO: closed triangle, PT: upward triangle, SP: closed square, SA: open square, PM: downward triangle, SB: x, HN: open diamond).

Species, NSC treatment, watering treatment, day*species and day*treatment were all important for explaining growth. The interaction between NSC treatment and day was removed from the model based on BIC (ΔBIC = -18.6), which indicates that NSC was not important for seedling growth. Growth was lower in the drought than in the water treatment ($\beta_{day*drought}$ = -0.05, 95% CI: -1.0 – -0.04).

Discussion

Within species variation in NSC concentrations showed that individuals with more stored NSC survived longer than individuals with less NSC. Furthermore, risk of mortality declined inversely with NSC concentrations across species and time to death increased with NSC concentrations. These results are consistent with previous work showing the benefit of having increased NSC concentrations for drought tolerance (Anderegg et al. 2012). However, NSC concentrations did not affect growth. Therefore, NSC concentrations explained variation in mortality risk both within and among species but were unimportant for growth regardless of water availability.

Although we used a controlled setting to examine the role of variable NSC concentrations in drought resistance, natural variation in carbon storage occurs from environmental and genetic mechanisms (Chapin et al. 1990; Hughes et al 2008). Water availability, nutrients, light and natural enemies all alter NSC concentrations (Kobe 1997; McDowell et al. 2011; Sala & Hoch 2009; Anderegg et al. 2012). For example, previous work on the same species found that individuals growing in high light had 3-fold greater NSC concentrations relative to conspecifics in low light

(Saner 2009). Furthermore, trees often accumulate NSC in drier sites relative to wetter sites (Sala & Hoch 2009; Anderegg et al. 2012). Additionally, species and genotypes naturally vary in functional traits such as carbon storage (Chapin et al. 1990; Choat et al. 2007; Hughes et al. 2008; Wiley & Helliker 2012). Therefore, consistent with our results, natural variability in individual NSC concentrations can promote population and community adaptation to altered climatic envelopes (Hughes et al. 2008; Buckley & Kingsolver 2012).

Shade and herbivory tolerance has been shown to correlate well with among species variability in NSC concentrations (Kobe 1997; Canham et al. 1999; Myers & Kitajima 2007), and it is not surprising that inter-specific differences in NSC concentration explain differences in resistance to drought (Fig 5). NSC stores play a role in maintenance of cell turgor and refilling of embolisms during drought (Hsiao 1973; Bucci et al. 2003). Species differences in resistance to drought, mediated by NSC concentrations, likely acts as a niche axis which explains species distributions in tropical environments (Engelbrecht et al. 2007).

Water availability significantly reduced growth, but NSC concentration was unable to buffer that effect. Furthermore, greater NSC concentrations did not allow faster growth of seedlings when water was non-limiting. This result may indicate that NSC storage was solely used as a buffer against drought stress and was not converted to structural growth when water was non-limiting. NSC storage is not likely a passive process, which occurs solely when there is a growth limitation, but an active process in competition with growth (Chapin 1990; Körner 2003; Sala et al. 2012; Wiley & Helliker 2012). However, relative to water availability NSC concentrations appear to have a negligible effect on growth.

Conclusions

Greater NSC concentrations both within and among species improve survival during drought. Individuals with large NSC reserves, from either a genotypic derived strategy towards greater storage or an environmentally imposed increase in storage, may persist if rainfall becomes more variable or declines under future climates. NSC storage may mediate the effects of climate change induced drought allowing adaptation to novel climate envelopes.

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Supplemental

Additional seedling monitoring

We recorded the date of leaf browning, decline in leaf angle, start of leaf loss and total loss of leaf. We made daily course measures of stomatal conductance (g_s) from 08:00 until 18:00 every 14 days with a steady state diffusion porometer (model SC-1, Decagon Devices, Inc., Pullman, WA, USA). During each daily course, we measured 120 seedlings over three hour periods (three individuals of each species in each NSC treatment within each watering treatment). Each of the 120 seedlings received three stomatal measurements per daily course (morning, mid-day and afternoon).

Test of differences after NSC treatment

We tested differences of within species seedling leaf area, height and diameter between Low-NSC and High-NSC treatments in order to ensure that seedlings were not statistically different in size prior to the drought treatment. We also compared non-structural carbohydrate concentrations (NSC) within species between Low-NSC and High-NSC treatments to ensure that greater NSC was achieved in the High-NSC treatment. All pre-treatment analysis used the nlme library (Pinheiro & Bates 2000) in the R statistical software package (2.15.1; R Core Team 2012).

Results of differences after NSC treatment

Non-structural carbohydrate (NSC) treatment and species were used to model leaf area, diameter, height and NSC, but their interaction did not explain any variability and was dropped. For all metrics of growth, species were significantly different in size (leaf area: $F_{9,88} = 9.7$, $p < 0.0001$; diameter: $F_{9,1378} = 538.2$, $p < 0.0001$ and height: $F_{9,1378} = 350.6$, $p < 0.0001$). However, differences in seedling size between NSC treatments were metric dependent. Leaf area was marginally significant

($F_{1,88}$ = 3.7, p = 0.06), diameter was non-significant ($F_{1,1378}$ = 0, p = 0.984) and height was significant ($F_{1,1378}$ = 15.0, p < 0.0001). As planned, NSC concentrations were significantly different between treatments and species (treatment: $F_{1,89}$ = 29.6, p < 0.0001 and species: $F_{9,89}$ = 7.3, p < 0.0001). NSC treatment explained more variability in NSC than species. Therefore, within a species seedlings had a similar size between NSC treatments (Fig 1) but were different in NSC concentrations (Fig 2).

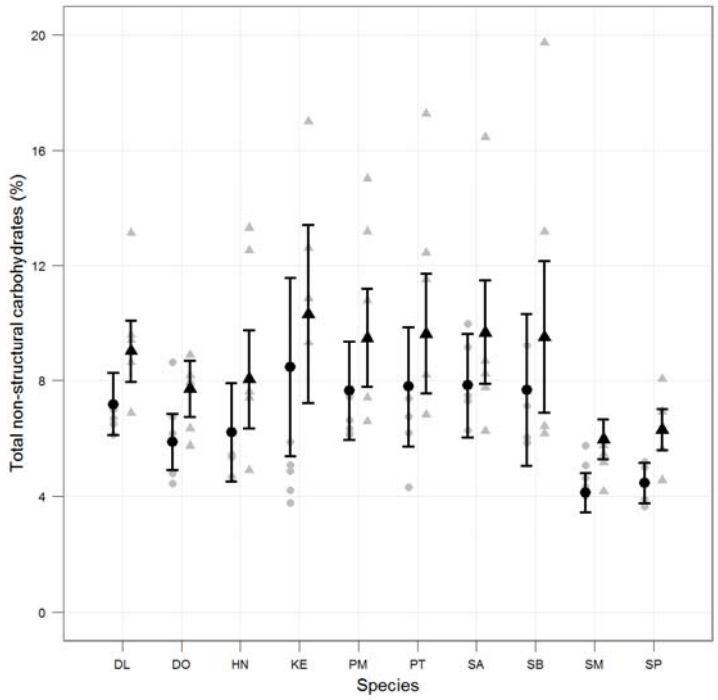

Fig S1 Non-structural carbohydrate concentrations for all ten species prior to drought treatments determined by linear mixed effects models. Triangles represent Low-NSC, and circles represent High-NSC. Grey points are observed data, and black points are means (±95% CIs).

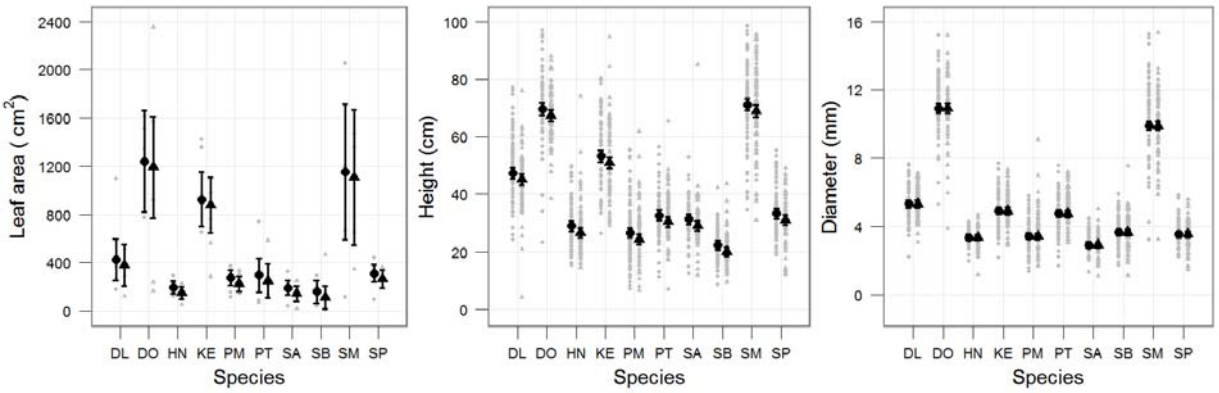

Fig S2 Size differences between low and high non-structural carbohydrate concentrations (NSC) for all ten species prior to drought treatments. Triangles represent Low-NSC, and circles represent High-NSC. Grey points are observed data, and black points are means (±95% CIs).

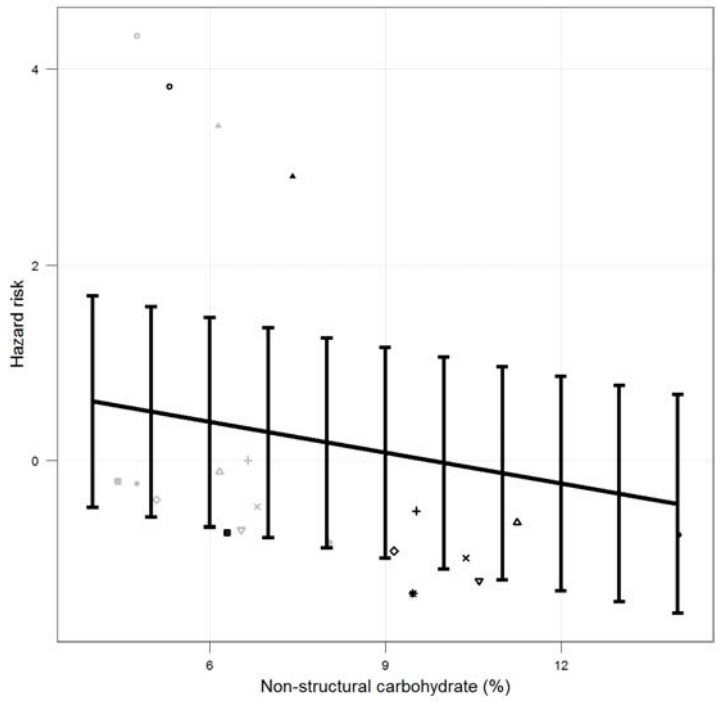

Fig S3 Hazard risk (extracted from the Cox proportional hazard model) modeled as a function of mean non-structural carbohydrate concentration of species from each NSC treatment. The line represents model fit with 95% CIs. Grey points represent mean values for each species from the Low-NSC treatment, and black points represent mean values for each species from the High-NSC treatment. (DO: cross, KE: x, SM: open circle, DL: closed square, PT: upward triangle, SP: closed diamond, SA: closed circle, PM: downward triangle, SB: closed triangle, HN: open diamond).

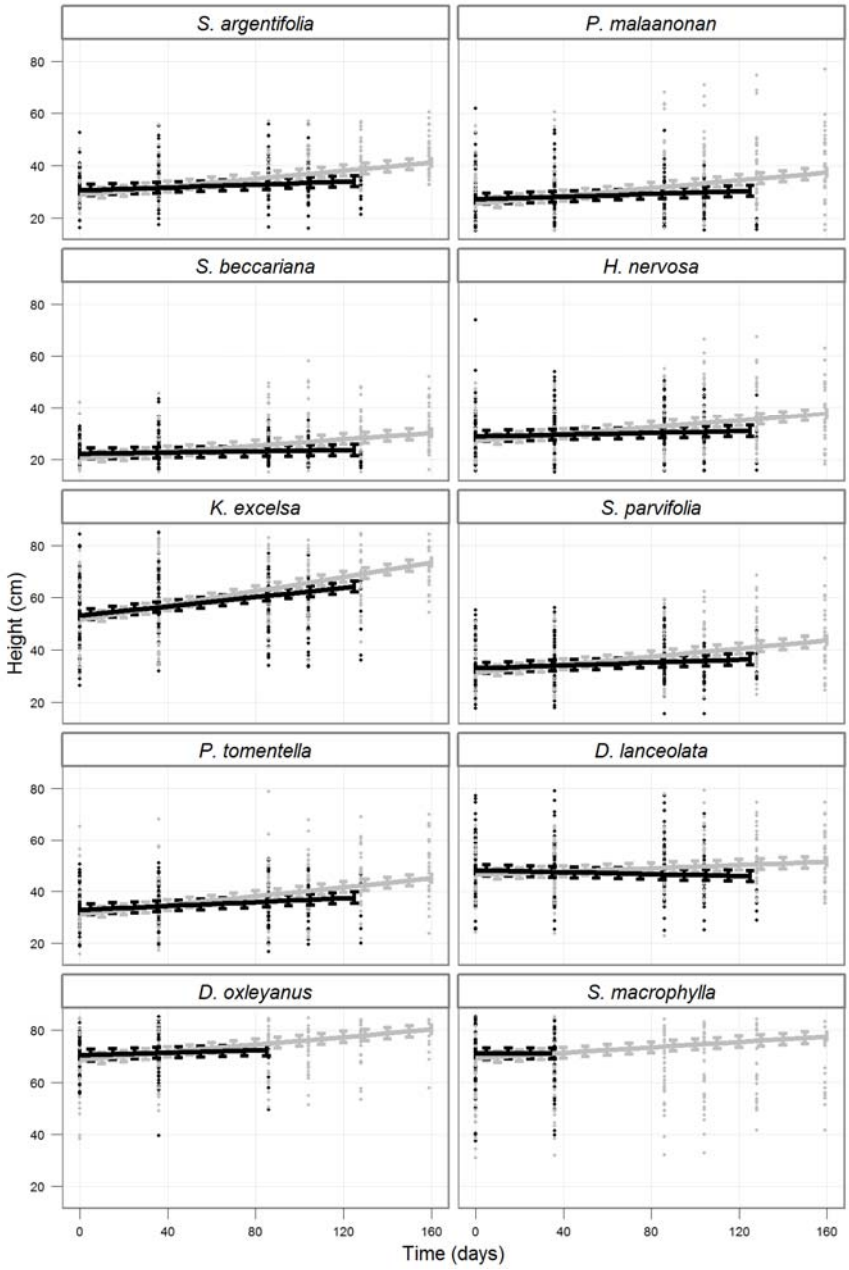

Fig S4 Height growth as a function of time since the start of the water and drought treatment. The lines represent model fits (±95% CIs) and points represent observations (grey: watered and black: drought).

General Discussion

In this thesis, I examine the importance of functional trait diversity of rain-forest trees in the recovery of primary forest species after logging and as insurance against climate change.

Logging and species coexistence

I found that seed dispersal in logged forest limited the recovery of forest diversity (Chapter 1). Time-scales on the order of centuries should be expected in severely logged forests for the recovery of diversity of the primary forest canopy trees. The irregular seed production and large seeds (> 500 mg), which have short dispersal distances, contribute to this slow forest recovery (Chazdon 2003; Brearley et al. 2007).

However, when seed dispersal from neighbouring trees occurred the processes that promote species diversity were functioning (Chapter 1) in that negative density-dependent mechanisms such as pathogens and invertebrate herbivory inhibited the establishment of the con-specific seeds of the nearby mature trees. However, as seedlings established the strength of negative density-dependent mechanisms declined. This decline in strength was likely a result of the short time-scale of the experiment as well as stochastic density-independent mortality events. Competition will likely play a more influential role in determining community composition, as establishing seedlings grow larger. Therefore, continued monitoring of the experimental plots will allow elucidation of the strength of intra- and inter-specific competition consistent with other tropical systems (Harms et al. 2000; Comita et al. 2010).

Drought and species coexistence

I showed that species range from sensitive to quite resistant in their response to drought (Chapter 2-4). The effect was dependent on the frequency of water (Chapter 2 and 3), the severity of the water deficit (Chapter 3), species traits (Chapter 3 and 4) and the ontogeny of the individual (Chapter 2-4). In order to highlight the importance of ontogeny, I will synthesize the results of the three drought studies for one species, *P. tomentella*.

In Chapter 2, the germination of *P. tomentella* was relatively insensitive to infrequent watering because days to germination increase only slightly from 13 days with daily water to 17 days under six-day watering cycles. However, after germination seedling biomass after 60 days of growth was 71% less in the six-day watering cycle than seedling biomass in the daily watering cycle. This decline in biomass was greater than all other species except *S. macrophylla*. In Chapter 3, the seedlings had recently dropped their cotyledons and were fully established autotrophs. At this stage, *P. tomentella* was tolerant to both infrequent watering and extreme water deficits relative to other species. Therefore, *P. tomentella* seedlings had a reversal from sensitive to tolerant between the establishing and established stage. *P. tomentella* seedlings adopted a conservative growth strategy by allocating biomass to root growth and storing high concentrations of non-structural carbohydrates. In Chapter 4, the seedlings were 10 months old. Although most traits were relatively similar between the two stages, leaf area had increased from 150 cm^2 to 350 cm^2. This resulted in a decrease of rooting depth per leaf area and in turn a more moderate tolerance to drought. This was driven by larger individual leaves (95.3 cm^2 in Chapter 4 versus 67.1 cm^2 in Chapter 3), which is consistent with recent reported correlations between leaf size and vulnerability to hydraulic failure (Scoffoni et al. 2011; Nardini

et al. 2012). Many dipterocarps appear to exhibit high ontogenetic variability in traits, and the results from these experiments should not be extrapolated to older age or larger size classes (Philipson, *personal communication*). Therefore, studies on the response of larger size classes to drought will be necessary to understand forest level effects beyond the seedling stage.

Readdressing the coexistence framework

The clear differential response among species demonstrates the potential importance of drought as a mechanism affecting species coexistence. However, it is unclear whether the observed functional trait differences will act as fitness differences leading to competitive exclusion or stabilizing niche differences leading to coexistence (HilleRisLambers et al. 2012). As a trade-off was observed between fast growth when water is non-limiting and improved survival when water is limiting (Chapter 3), I hypothesize that drought is more likely to act as a stabilizing mechanism preventing competitive exclusion of slower growers when water is a limited resource. Increased frequency and severity in drought could lead to increased competition for water which may usurp competition for light which is the limiting resource under average rainfall conditions.

Assumptions and future research

Some general assumptions were made in the methodology and interpretation of the results of this thesis, and as with many studies, this work has identified gaps in our understanding of tropical forest ecology and species response to drought. Three caveats regarding methodology and interpretation arose from this thesis. First, root growth is an important adaptation for survival during drought (Sperry et al. 2002; Markesteijn & Poorter 2009), but the use of pots may hinder root growth causing

biologically inaccurate results in mortality rates. Second, I assume that tolerance to drought (measured by survival or reduced dieback) provides an individual or species with a competitive advantage when water becomes available (MacGillivray et al. 1995; Whitford et al 1999). Finally, if niche differences are assumed from among species trait differences, then species must vary along a trait gradient (McGill et al. 2006; Kraft et al. 2008; Jung et al. 2010). However, if intra-specific variation is larger than inter-specific variation, then observed niche differences are exaggerated (Albert et al. 2011; Violle et al. 2011). From these caveats, I pose three specific future research questions:

1) What is the potential root growth of these species in natural settings?

2) Does resistance to drought equate to resiliency after drought?

3) Is among species variability greater than within species variability for tree species of Borneo?

Specific experiments are necessary to answer these questions. However, I address these three questions with some initial analysis from additional experiments and compiled data sets.

Root Growth

I tested root growth potential by using an experimental manipulation within a forest setting. I planted one meter long PVC piping 40 cm into the soil in forest gap and understory plots. The pipe was filled with local soil, and the below-ground section of pipe was drilled with holes to allow interaction with the surrounding soil. Forty eight one-year old seedlings from each of these five species were planted into the pipes. Species were selected based on their distinct growth strategies and traits.

This design allowed seedlings to have uninhibited vertical root growth while allowing easy extraction of the entire root at harvest. Root depth at the end of nine months of growth was surprisingly shallow for dipterocarps. Alternatively, *K. excelsa* and *D. oxleyanus* had rooting depths greater than 40 cm (Fig 1). Additionally, biomass growth partitioned by 10 cm increments showed that dipterocarps allocate more to surface roots through time (Fig 2). This provides preliminary evidence that the pots did not inhibit dipterocarps while *K. excelsa* and *D. oxleyanus* were potentially pot bound. However, this likely was unimportant in my experiments because the longest root was measured in all experiments; it therefore could potentially be longer than the pot depth.

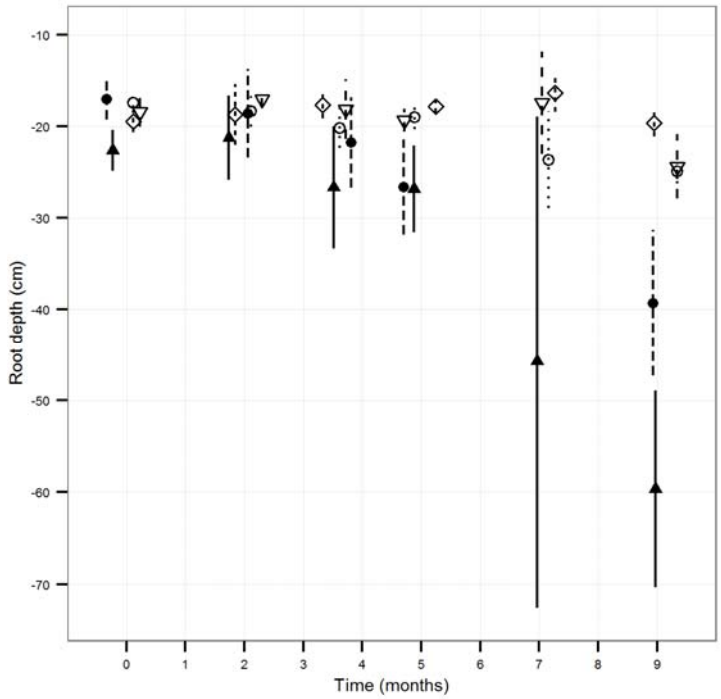

Fig 1 Mean root depth extension (±95% CI) through time for five species. Open symbols represent dipterocarps (*H. nervosa* - open diamond, *P. malaanonan* - downward triangle and *S. macrophylla* - open circle), and closed symbols represent non-dipterocarps (*D. oxleyanus* – upward triangle and *K. excelsa* - circle). Points are jittered for readability.

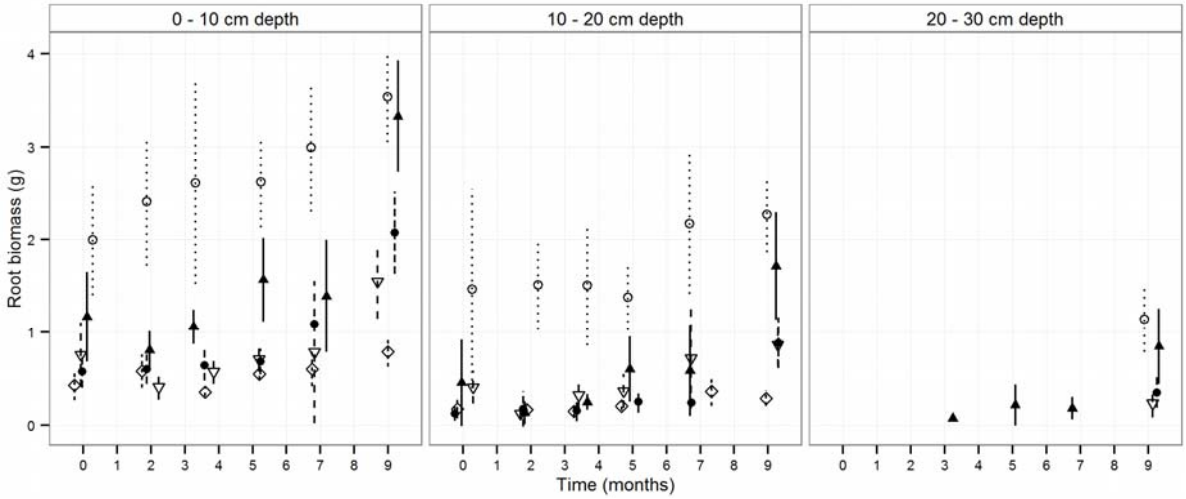

Fig 2 Mean root biomass (±95% CI) through time for five species at 10 cm increments to a total depth of 30 cm (0 – 10 cm, 10 – 20 cm and 20 – 30 cm). Open symbols represent dipterocarps (*H. nervosa* - open diamond, *P. malaanonan* - downward triangle and *S. macrophylla* - open circle), and closed symbols represent non-dipterocarps (*D. oxleyanus* – upward triangle and *K. excelsa* - circle). Points are jittered for readability.

Resistance vs. Resilience

I tested recovery from drought in the forest using 12 paired-plots of communities of 20 species. Paired-plots consisted of a control with natural rainfall (controls were watered if there was no rain for 3 consecutive days) and a drought plot, which was covered with clear plastic sheeting suspended by wire 1.5 m above the seedlings. Additionally, a small aluminum barricade (5 cm above and below the soil surface) was placed upslope from the drought plots to prevent surface flow into the plots. A three-month drought was simulated approximately following the rainfall pattern from Feb. – Apr. of 2010. At the end of the drought, leaf area was estimated for each individual from leaf length and width measurements. The same measurements were made 1 month after the drought and again 4.5 months after the drought. From these measurements I could assess dieback as the leaf area difference

between drought and control seedlings relative to control leaf area for each species at the end of the drought (Fig S1). The slope of the growth curve of the drought seedlings of each species was used as the metric for resiliency. If resistance equates to greater resilience, then there should be a negative correlation between dieback and growth rate (Fig 3). Size-corrected growth rate was negatively correlated with percent dieback (Spearman correlation coefficient = -0.45, p =0.04). Growth rate declined by -0.0001 (95% CI: -0.0002 – 0.00003) with every 1% increase in dieback. Therefore, the assumption that resistance relates to resilience is valid. Drought tolerant species have the benefit of improved survival during drought as well as faster recovery from drought, which would likely lead to a competitive advantage.

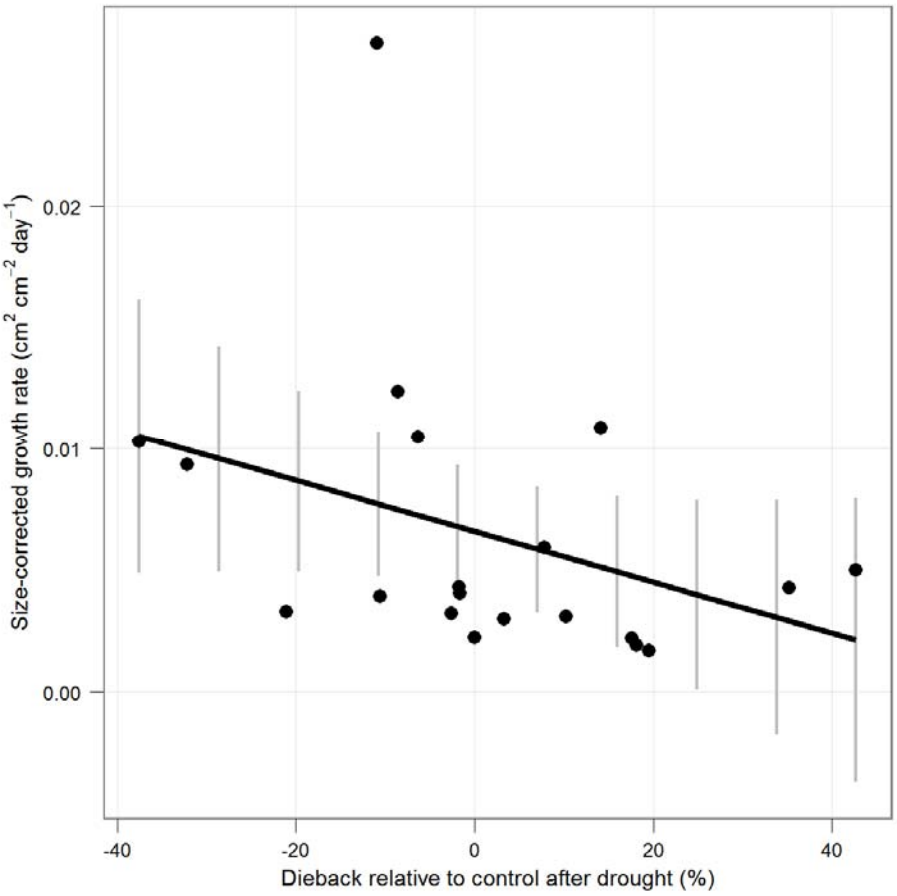

Fig 3 Size-corrected growth rate (±95% CI) of each species following a drought (resiliency) as a function of relative dieback of seedlings under drought for each species (resistance).

Trait variance

In an effort to test within and among species variance a database of traits from multiple studies on primary forest tree species was compiled. However, the method of determining within and among species variance is not clear. One potential method would be to calculate the variance components to identify the relative importance of different explanatory variables (Gelman & Hill 2007; Hector et al. 2011). I test this method for specific leaf area, root mass fraction, rooting depth to leaf area and seed mass. For each of these traits, I define a model with a fixed effect that estimates the global mean of the trait, and random effects for individual (within species variance), species (among species variance) and dataset (account for inherent in experiments). I perform all analysis using the lme4 library (Bates & Maechler 2011). The results show that the strength of within versus among variance is trait dependent (Fig 4). Among species variance is important for root mass fraction, seed mass and specific leaf area, but within species variance has importance for specific leaf area as well. Within species variance is important for root depth to leaf area. This method is not perfect, and it needs to be improved in order to better assess the importance of within and among variability. However, it does identify an important point: trait selection is important for identifying among species partitioning, as some traits differ more among species within (Albert et al. 2011).

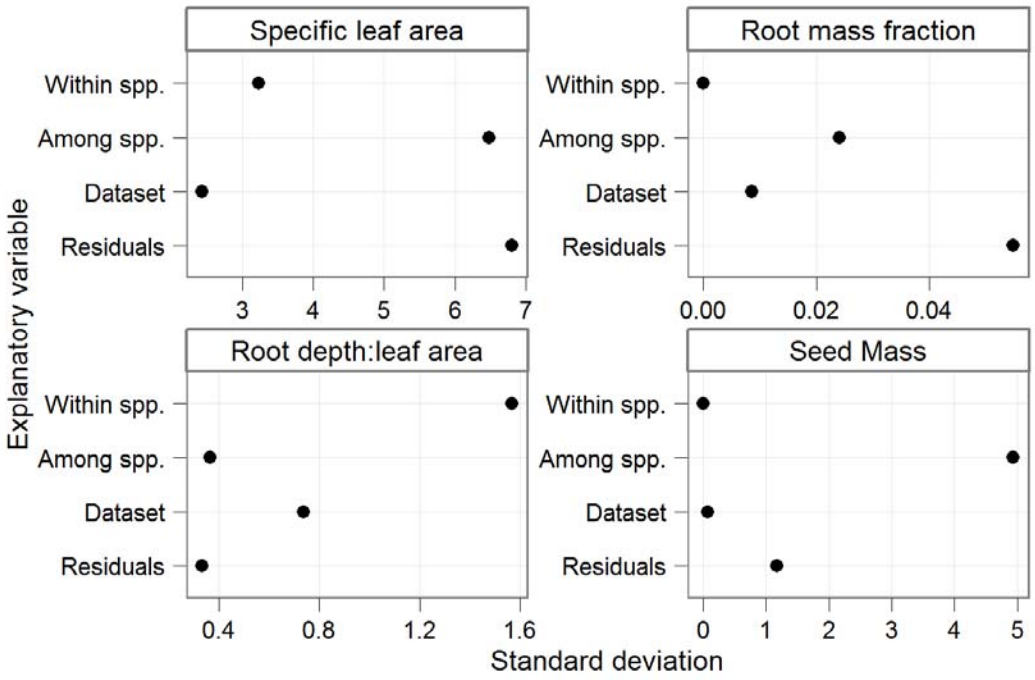

Fig 4 The variance components (standard deviation) of four explanatory variables for four different traits.

Conclusions

In this thesis, I show that the primary forest tree species of Borneo clearly vary in their tolerance to drought. These results are consistent with results from other tropical forests throughout the world. Therefore, this important ecosystem has the potential to adapt to climatic changes. However, the rate and intensity of climate change is still unknown, and immense uncertainty remains regarding the future survival of the plant species of Borneo.

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Supplement

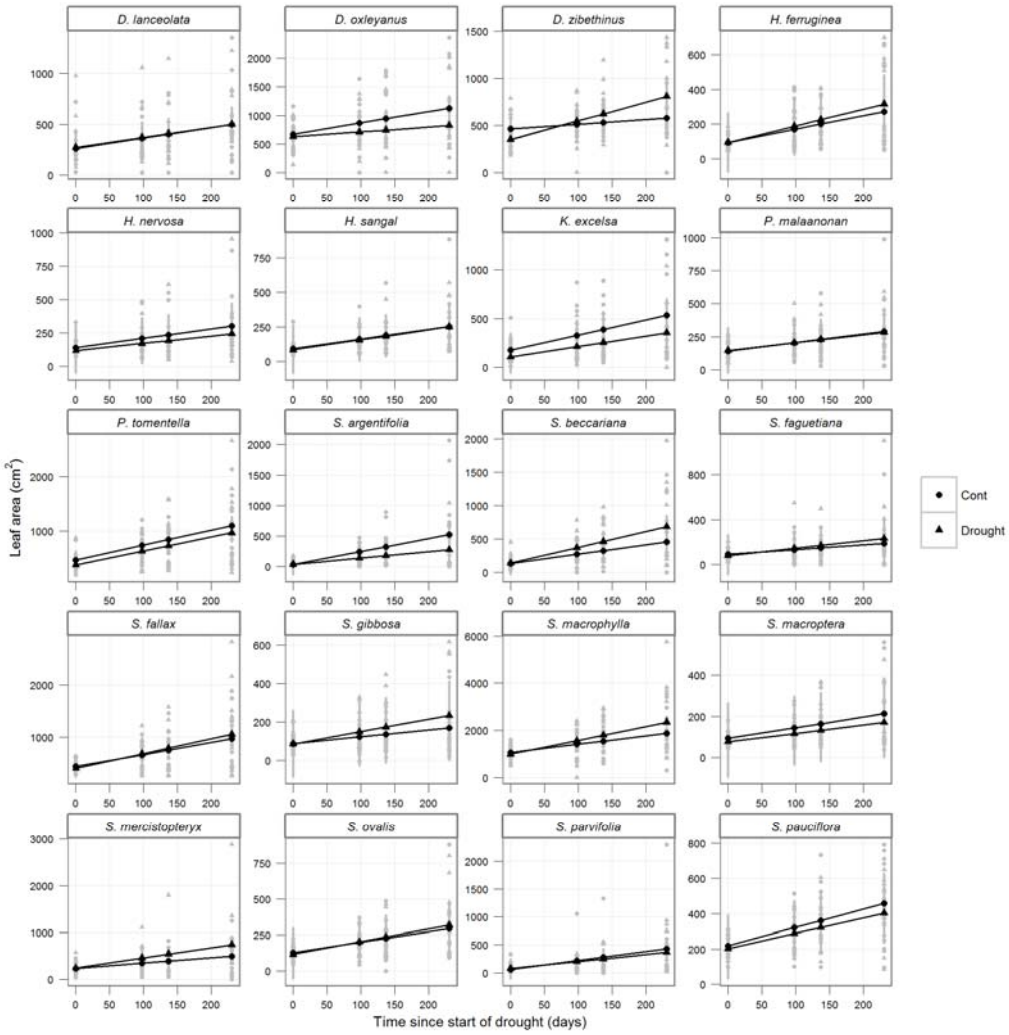

Fig S1 Leaf area as a function of time since the start of drought. Lines and black points are model estimates, and grey points are measured leaf area.

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Curriculum Vitae

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Annex

The Sabah Biodiversity Experiment: a long-term test of the role of tree diversity in restoring tropical forest structure and functioning

Andy Hector, Christopher Philipson, Philippe Saner, Juliette Chamange, Dzaeman Dzulkifli, Michael O'Brien, Jake Snaddon, Philip Ulok, Maja Weilenmann, Glen Reynolds & Charles Godfray. *The Philosophical Transactions of the Royal Society B*, 366, 3303-3315, 2011

Germination and establishment of dipterocarp seedlings: seedling recruitment of three dipterocarp species in logged lowland rainforest, Sabah, Borneo (Malaysia)

Roman Kellenberger**,** Practical Report
(Michael O'Brien advised data collection and analysis for BSc thesis)